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High Prevalence of Rodent-Borne *Bartonella* spp. in Urbanizing Environments in Sarawak, Malaysian Borneo

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Abstract. Rodents are the most prominent animal host of *Bartonella* spp., which are associated with an increasing number of human diseases worldwide. Many rodent species thrive in urban environments and live in close contact with people, which can lead to an increased human risk of infection from rodent-borne pathogens. In this study, we explored the prevalence and distribution of *Bartonella* spp. in rodents in urban, developing, and rural environments surrounding a growing city in Sarawak, Malaysian Borneo. We found that although *Bartonella* spp. infection was pervasive in most rodent species sampled, prevalence was highest in urban areas and infection was most commonly detected in the predominant indigenous rodent species sampled (*Sundamys muelleri*). Within the urban environment, parks and remnant green patches were significantly associated with the presence of both *S. muelleri* and *Bartonella* spp., indicating higher localized risk of infection for people using these environments for farming, foraging, or recreation.

The genus *Bartonella* contains a diverse group of emerging, zoonotic, gram-negative, facultative intracellular *Alphaproteobacteria* that infect a wide range of wildlife and domestic animals. Rodents appear to be the most common wildlife host of *Bartonella*, having been associated with 20/33 described species to date, many of which have also been linked to disease in people.[1,2] Transmission between animal hosts appears to be primarily through ectoparasite vectors, including fleas, mites, and ticks.[2] However, it is clear from the diversity of recently described *Bartonella* spp. associated with a widening range of mammalian hosts and potential arthropod vectors, that the ecology of these bacteria is complex and not well understood.[3,4]

In people, infection with *Bartonella* often results in undifferentiated febrile illnesses that may be similar in clinical presentation to those caused by other pathogens (e.g., *Borrelia* spp.).[2,5] This suggests that the global burden of *Bartonella*-associated diseases, although significant, may be underestimated. Some groups of people (e.g., outdoor workers, immunocompromised people, and the homeless) may be particularly vulnerable to infection, indicating that human behavior and local ecology may be significant contributors to zoonotic disease risk.[2] In this study, we screened indigenous and invasive rodents found in urban, developing, and rural locations around the city of Kuching, Sarawak, for *Bartonella* spp., to begin to explore the role of local ecology in the presence and prevalence of *Bartonella* in Malaysian Borneo.

As part of a larger study on the effect of urbanization on rodent-borne diseases, we collected 316 rodents from several sites in urban, developing, and rural areas in and around the city of Kuching, Sarawak, between September 2015 and April 2016 (Supplemental Figure 1). The predominate land use type at each site was characterized by estimating the proportion of green or gray space within 10 m and 100 m radii from the trapping site and by the proportion of forest cover. Mean forest cover was estimated using QGIS v 2.14.0 (2018) and previously published forest cover and loss datasets at the Landsat pixel scale, ranked and grouped into tertiles, which were categorized as minimal, moderate, or maximal forest cover (https://earthenginepartners.appspot.com/science-2013-global-forest). Rodents were live-trapped using locally made wire-mesh traps and euthanized by over-anesthetization in isoflurane, followed by bilateral thoracotomy. Tentative species assignment, sex, breeding status, and body mass (as a proxy for age) were recorded, and tissues and ectoparasites (i.e., mites, lice, fleas, and ticks) were collected and frozen directly on dry ice. The species identity of each animal was confirmed using primers BatL5310 and R6036R, which amplify ~750 bp of the cytochrome oxidase I gene.[6] Based on the resultant sequences, rodents grouped with eight species from four genera, with most individuals falling within the *Rattus rattus* super-group ($N = 187$) or classified as *Sundamys muelleri* ($N = 100$) (Supplemental Table 1). Although three species of the *R. rattus* super-group were delineated by this method (i.e., *Rattus* sp. R3, *Rattus tanezumi*, and *Rattus tiomanicus*), we considered them collectively for this analysis, as distinct mitochondrial lineages of this super-group are known to hybridize when sympatric.[7,8] All animals and samples were collected with permission from the Commonwealth Scientific and Industrial Research Organization Australian Animal Health Laboratory Animal Ethics Committee (#1750) and the Sarawak Forests Department (#NCCD.907.4.4(JLD.12)-131).

DNA was extracted from ~30 mg of rodent spleen homogenate using the AllPrep DNA/RNA mini Kit (Qiagen Inc., Valencia, CA) and subjected to a nested polymerase chain reaction (PCR) targeting the *Bartonella* citrate synthase A (*glt*A) gene using a nested PCR reaction. Positive samples generated either a 767-bp product in round 1 (primers CS443f and CS1210r) or a 694-bp product in round 2 (primers CS443f and BhCS.1137n) of the PCR and were confirmed by Sanger sequencing.[9,10] The resultant sequences (GenBank accession nos. MG807665–MG807845) were trimmed for quality and length and were manually aligned with those of a representative sample of *Bartonella* spp. in Geneious version 10.2.2.[11] A maximum likelihood (ML) phylogenetic tree was constructed using the Generalized Time Reversible plus gamma model of nucleotide substitution in PhyML v3.1, with 1,000 bootstrap replications.[12] Sequences were then trimmed to include only the 327-nt region of *gltA* (positions

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801–1127) commonly used for taxonomic classification and compared with publicly available sequences from verified *Bartonella* species.[13] For downstream analyses, sequences were considered to belong to a known species if they shared ≥ 96% sequence similarity and clustered with the respective species-specific clade in the ML tree (Figure 1). Statistical analyses were performed to identify significant relationships between infection status and biological and environmental

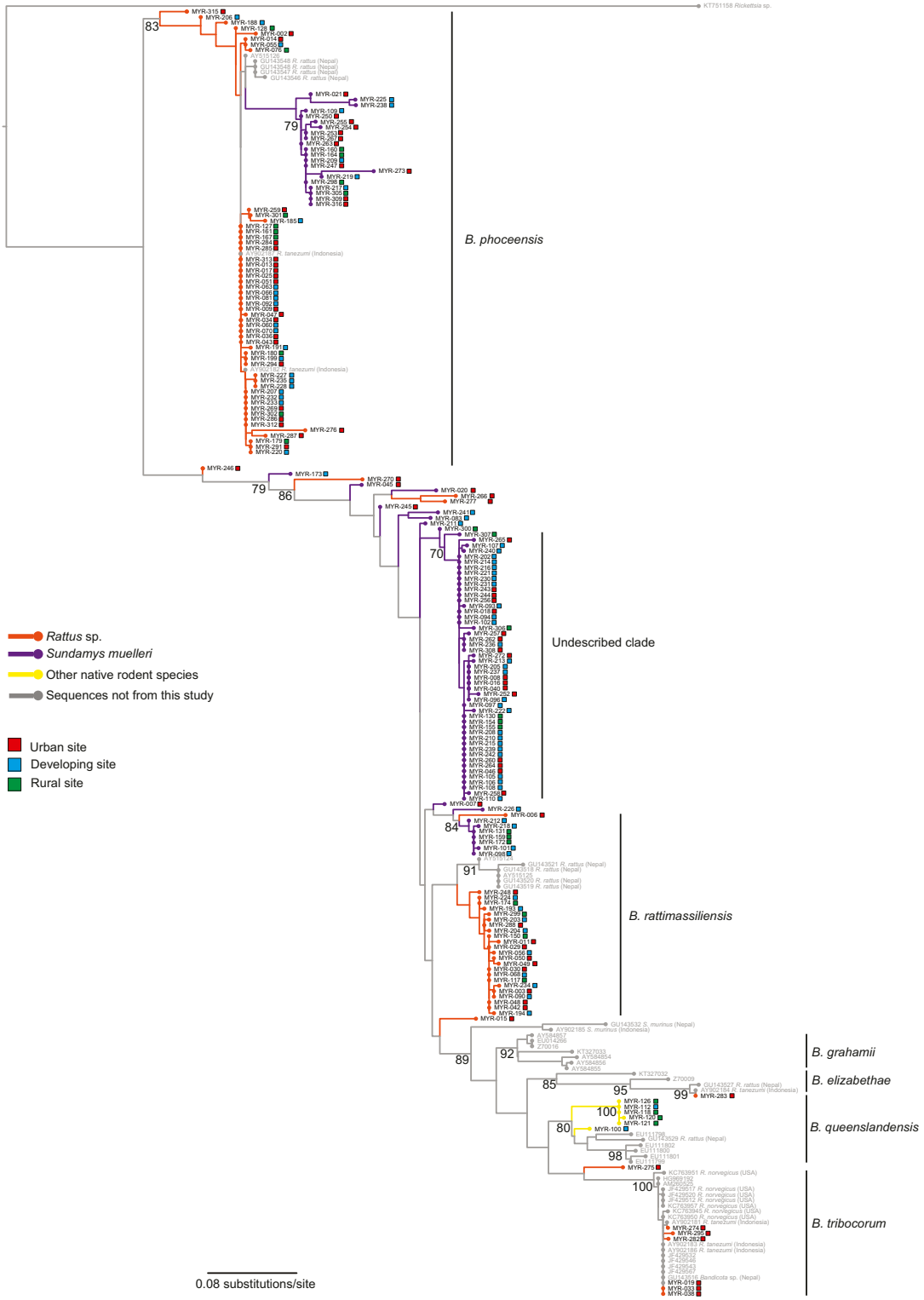

FIGURE 1. Maximum likelihood phylogeny of a 641-nt region of the citrate-synthase A gene of *Bartonella*. Dataset includes 181 *Bartonella* spp. detected in native and invasive rodent species in and around Kuching, Malaysia, and selected publicly available sequences of verified *Bartonella* species and those from similar studies. Bootstrap values are shown for nodes with > 70% support. Names of the sequences determined in this study are indicated in black (e.g., MYR-300), and both the associated host species and location of each site are indicated. When previously published sequences are included but not associated with a *Bartonella* species, the host and country of origin are shown.

variables and considered all *Bartonella* together, as well as each of the three predominant groups separately. Standard tests were used including the Fisher's exact test (variables with two outcomes), Chi-square test of independence (variables with more than two outcomes), and *Z* test (measurement variables).

A total of 181 animals (57.3%) were positive for *Bartonella* spp., including 47.1% of *Rattus* sp. ($N = 88$) and 87.0% of *S. muelleri* ($N = 87$) (Supplemental Table 1). *Sundamys muelleri* were more likely to be infected with *Bartonella* than *Rattus* sp., and this difference was statistically significant (Table 1, $P < 0.01$). *Bartonella* spp. prevalence also varied by location, with rodents from urban and developing regions more likely to be infected than rodents from rural areas (Table 1, $P < 0.01$), and by body mass (Table 1, $P < 0.01$), but not by gender. Although heavier (older) rodents were more likely to be infected than lighter (younger) rodents, this difference was significant only when individuals from the genus *Rattus* were considered separately. *Bartonella* spp. prevalence was also found to vary by site type, with predominantly green sites more likely to harbor infected individuals than gray sites (Table 1, $P < 0.01$). The presence of some types of ectoparasite also correlated with *Bartonella* spp. infection, as individuals with mites or lice were more likely to be positive for *Bartonella* than those without (Table 1, $P < 0.05$).

Sequence and phylogenetic analyses revealed the presence of six distinct groups of *Bartonella* in rodents, five of which were closely related to verified *Bartonella* species (Figure 1). Six sequences were identified by both methods as belonging to *Bartonella queenslandensis*, six to *Bartonella tribocorum*, one to *Bartonella elizabethae*, 31 to *Bartonella rattimassiliensis*, and 72 to *Bartonella phoceensis* (Supplemental Table 2). A further 48 sequences were < 96.0% similar to previously described species, yet clustered tightly together in the ML tree and were > 96.0% similar to each other (henceforth referred to as the "undescribed clade"). The final 18 sequences fell outside of the major clades in the ML tree and/or were unassigned by % sequence similarity (Figure 1). Differences in the distribution of the three predominant *Bartonella* clades were observed, as members of the undescribed clade were only detected in *S. muelleri* trapped in green sites and were more prevalent in sites with more forest cover, as well as in urban and developing locations (Figure 2). Positive associations were also found between the prevalence of *B. rattimassiliensis* and the presence of lice ($P < 0.05$) and the unassigned *Bartonella* and the presence of ticks ($P < 0.01$), whereas *B. phoceensis* was not positively associated with the presence of any ectoparasites ($P > 0.05$) (Table 1).

In this study, we found that *Bartonella* spp. infection was pervasive in both indigenous and invasive rodents and across rural, developing, and urban locations. *Bartonella* spp. prevalence was highest among rodents from urban sites and lowest among those from rural areas (Figure 2, $P < 0.01$). Notably, 85% and 100% of *S. muelleri* trapped at urban and developing sites, respectively, were *Bartonella* spp. positive. In these locations, *S. muelleri* rodents were restricted to remnant green patches, where they were observed to exist at a higher density than in rural environments. Therefore, high-density populations of rodents restricted to relatively small habitat patches may experience increased rates of intraspecies contact, facilitating *Bartonella* transmission.

It is unclear how the increased prevalence of *Bartonella* in rodent populations in urban and urbanizing environments might influence the risk of zoonotic transmission for people in cities; however, human infection with *B. elizabethae*, *B. tribocorum*, and *B. rattimassiliensis* have been documented, and all three of these species have been associated with urban rodents.[14–16] Human risk of infection is likely heterogeneous in cities and governed in part by the intensity of contact between rodents, their ectoparasite vectors, and people.[17] In particular, the frequent use of remnant green patches by people in and around Kuching for foraging and recreation may, therefore, increase risk of zoonotic infection, particularly from tick or flea-borne *Bartonella*.

TABLE 1

Statistical tests of the associations between *Bartonella* spp. infection and environmental or ecological variables

Variable	Test	df	Statistic	Significance
Rodent species (*Rattus*/*Sundamys*)	Fisher's exact			
Overall		1	$P = 7.37 \times 10^{-12}$	$P < 0.01$
Undescribed clade only		1	$P = 3.55 \times 10^{-26}$	$P < 0.01$
Mites (presence/absence)	Fisher's exact			
Overall		1	$P = 1.06 \times 10^{-3}$	$P < 0.01$
Rattus spp. only		1	$P = 0.011$	$P < 0.05$
Lice (presence/absence)	Fisher's exact			
Overall		1	$P = 3.76 \times 10^{-3}$	$P < 0.01$
Rattus spp. only		1	$P = 0.016$	$P < 0.05$
Bartonella rattimassiliensis only			$P = 0.025$	$P < 0.05$
Ticks (presence/absence)	Fisher's exact			
Undescribed clade only		1	$P = 0.001$	$P < 0.01$
Location (urban/developing/rural)	χ^2			
Overall		2	$\chi^2 = 16.992$	$P < 0.01$
Undescribed clade only		2	$\chi^2 = 12.466$	$P < 0.01$
Site type (gray/green)	Fisher's exact			
Overall		1	$P = 5.35 \times 10^{-8}$	$P < 0.01$
Undescribed clade only		1	$P = 3.30 \times 10^{-13}$	$P < 0.01$
Forest cover (min/mod/max)	χ^2			
Undescribed clade only		2	$\chi^2 = 19.923$	$P < 0.01$
Weight	*Z*-test			
Overall		–	$Z = 78.730$	$P < 0.01$
Rattus spp. only		–	$Z = 43.075$	$P < 0.01$

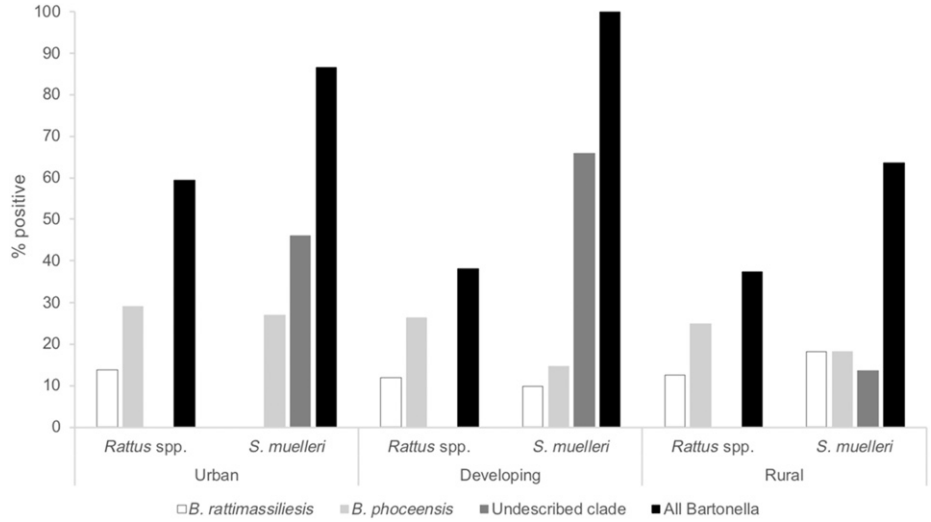

FIGURE 2. Proportion of *Rattus* sp. and *Sundamys muelleri* rodents infected with the three predominant groups of *Bartonella* identified in this study.

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