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Rats and the city: Implications of urbanization on zoonotic disease risk in Southeast Asia

Kim R. Blasdell[a], Serge Morand[b,c], Susan G. W. Laurance[d], Stephen L. Doggett[f], Amy Hahs[g], Kelly Trinh[d,e], David Perera[h], and Cadhla Firth[i,1,2]

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Urbanization is rapidly transforming much of Southeast Asia, altering the structure and function of the landscape, as well as the frequency and intensity of the interactions between people, animals, and the environment. In this study, we explored the impact of urbanization on zoonotic disease risk by simultaneously characterizing changes in the ecology of animal reservoirs (rodents), ectoparasite vectors (ticks), and pathogens across a gradient of urbanization in Kuching, a city in Malaysian Borneo. We sampled 863 rodents across rural, developing, and urban locations and found that rodent species diversity decreased with increasing urbanization—from 10 species in the rural location to 4 in the rural location. Notably, two species appeared to thrive in urban areas, as follows: the invasive urban exploiter *Rattus rattus* ($n = 375$) and the native urban adapter *Sundamys muelleri* ($n = 331$). *R. rattus* was strongly associated with built infrastructure across the gradient and carried a high diversity of pathogens, including multihost zoonoses capable of environmental transmission (e.g., *Leptospira* spp.). In contrast, *S. muelleri* was restricted to green patches where it was found at high densities and was strongly associated with the presence of ticks, including the medically important genera *Amblyomma*, *Haemaphysalis*, and *Ixodes*. Our analyses reveal that zoonotic disease risk is elevated and heterogeneously distributed in urban environments and highlight the potential for targeted risk reduction through pest management and public health messaging.

urbanization | zoonotic diseases | Southeast Asia | rodents | land-use change

Significance

Urbanization is rapidly transforming Southeast Asia, altering the landscape and the interactions between people, animals, and the environment. These changes have the potential to exacerbate many existing health challenges in the region, including those posed by zoonoses. Here, we used a novel, multidisciplinary, ecosystem-level approach to examine the influence of urbanization on zoonotic disease risk in a Southeast Asian city. We infer that urbanization alters the ecology of animal reservoirs, arthropod vectors, and pathogens in a manner that may increase transmission risk from multiple zoonotic diseases in urban areas. This effect was particularly strong for pathogens associated with environmental or tick-borne transmission, providing targets for the development of low-cost interventions to reduce zoonotic disease risk in tropical cities.

Urbanization is a widespread and significant process of global change that modifies the landscape rapidly, extensively, and often permanently. These environmental changes are accompanied by a marked reduction in biodiversity in cities that is driven by varied responses from plant and animal species (1). Many species are highly sensitive to the effects of urbanization—particularly the loss and fragmentation of suitable habitats—and may disappear from the urban environment completely. Nonetheless, the process of urbanization also creates new environmental conditions and an abundance of novel resources. These enable some wildlife (termed urban exploiters) to be highly successful in cities, where they can be found at unusually high densities (2).

Several rodent species are among the most successful urban dwellers. These include the ubiquitous commensals *Mus musculus* (the house mouse), *Rattus norvegicus* (the Norway rat), and the *Rattus rattus* species complex (the black rat and its relatives), which are thought to have coexisted with people for thousands of years. Recently, it has been argued that these urban exploiters are now so well adapted to cities that they should be considered native wherever they are found in the urban environment (3). As urbanization has intensified globally, an increasing number of rodent species also appear to be adapting to local city environments (termed urban adapters), where they are frequently found within both planned and remnant green spaces (3–5). Unfortunately, rodent presence in cities can be both an economic burden and a threat to human health (6, 7). Urban rodent infestations have been associated with an increased risk of asthma and allergies, as well as with the spread of a range of zoonotic and food-borne illnesses, including leptospirosis, hantavirus, Lassa fever, *Salmonella enterica*, and vector-borne bacterial infections, such as scrub typhus and plague (7–11). However, little is known about the drivers of rodent-borne zoonotic disease transmission in cities or how we might reduce the impact of urban rodents on human health through changes to infrastructure, resource distribution, or human behavior.

Tropical cities often bear the brunt of rodent-associated problems, with informal infrastructure and limited sanitation combining to create favorable environments for rodents, while warmer temperatures and increased humidity act to improve the environmental survival and transmissibility of some of the pathogens they carry (7, 10, 12). In addition, many arthropod vectors of rodent-borne diseases thrive under tropical conditions, including ticks, mites, and mosquitoes, which may further increase human

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[1]To whom correspondence may be addressed. Email: firth@ecohealthalliance.org.

[2]Present address: EcoHealth Alliance, New York, NY 10018.

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disease risk (10, 13, 14). Critically, ~90% of urban population growth in the next 30 years is expected to occur in lower- and middle-income countries in tropical and subtropical Asia and Africa (15). This rapid growth has the potential to exacerbate many of the public and environmental health challenges that exist in these regions, including those posed by rodents. Unfortunately, research into the ecology of urban rodents and associated zoonoses has been biased toward temperate cities, with preliminary investigations into rodent-borne pathogens in the tropics available for only a handful of cities, including Bangkok, Kuala Lumpur, Morogoro, and Salvador (16–20).

Despite the increasing relevance of urban ecosystems to human health in the tropics, little is known about how rodents use urban and periurban landscapes, the features of the environment that promote their persistence, or how the process of urbanization is likely to affect human disease risk. In this study, we explored how the abundance and diversity of rodents and their ectoparasites varied across a gradient of urbanization (Fig. 1) surrounding a tropical city in Malaysian Borneo (Kuching, Sarawak) and examined the environmental features associated with their presence. To begin to understand how urbanization might impact zoonotic disease risk in tropical cities, we further examined the prevalence of select groups of rodent-borne microbes in sampled animals across the urban–rural gradient and discuss how urbanization may affect human disease risk.

Results

Rodents and the Urban–Rural Gradient. Our initial field-based classification of 115 study sites into rural, developing, or urban categories largely concurred with our postsampling geographic information system (GIS)–based site classification, confirming that our a priori designation of landscape types based on urban development history also largely reflects a gradient of contemporary urban land cover (see *Methods* for details). This gradient was comprised of 65 rural sites encompassing dispersed villages and natural green spaces (collectively referred to as the "rural location"), 23 developing sites where urbanization is currently underway (i.e., the "developing location"), and 27 established urban sites comprised of green and gray spaces throughout the city of Kuching (i.e., the "urban location"). Throughout this manuscript, the term location is used to refer to the collection of sites that cluster together ($n = 3$; urban, developing, or rural locations), while the term site refers to a single sampling site that is nested within a location ($n = 115$).

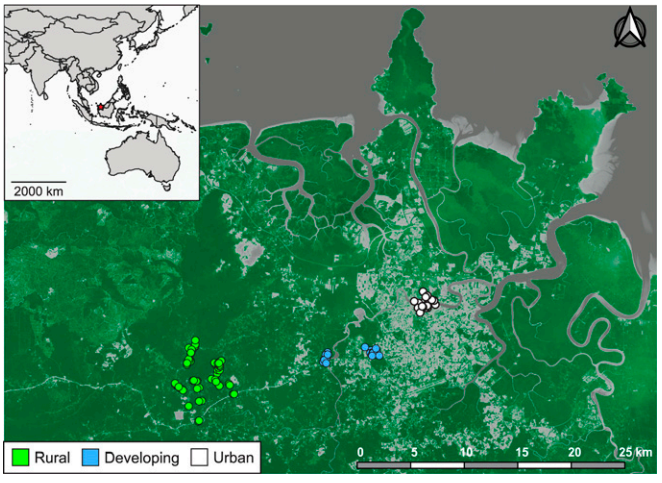

Fig. 1. Sites of rodent collection across the urban–rural gradient. From left to right: rural (green circles; Mount Singai), developing (blue circles; Batu Kawa district), and urban (white circles; Central Kuching) locations are shown. The background imagery shows the NDVI layer used to characterize the variability in land cover for each site; pixels with full vegetation cover are shown in green, built surfaces and bare soils are shown in light gray, and water and other land cover features with negative NDVI values are shown in darker gray. The *Inset* in the upper left corner indicates the location of the study—Sarawak, Malaysia, with the city of Kuching marked by a red star.

Across all sites, 863 rodents were captured over 7,665 trap-nights. We collected 246 rodents from the rural location, 318 from the developing location, and 299 from the urban location (*SI Appendix*, Table S1). These comprised individuals from 10 species, with the majority (92%) assigned to four species, as follows: *R. rattus* lineage R3 ($n = 375$), *Sundamys muelleri* ($n = 331$), *Rattus tiomanicus* ($n = 54$), and *R. rattus* lineage *tanezumi* ($n = 41$). These four species were also the only species collected across the entire urban–rural gradient. There was a clear decrease in rodent species richness with increasing urbanization (*SI Appendix*, Fig. S1); a total of 10 species were sampled in the rural location (Jack1 estimator = 16.7 ± 8.0; bootstrap estimator = 20.0 ± 12.9), 7 in the developing location (Jack1 = 11.7 ± 5.7; bootstrap = 9.1 ± 3.2), and 4 in the urban location (Jack1 = 6.7 ± 3.1; bootstrap = 5.2 ± 1.8). However, rodent community composition was highly similar between the rural and developing locations (Beta diversity, $\beta = 0.824$), with decreasing similarity between developing and urban locations ($\beta = 0.730$) and urban and rural locations ($\beta = 0.571$). Across all species, slightly more males (51.9%) than females were trapped; however, these differences were not significant for the four most abundant species collected (*SI Appendix*, Table S1, $P > 0.05$). For all species, the vast majority of rodents captured were adults, with the highest proportion of juveniles belonging to the species *R. rattus* lineage *tanezumi* (14.6%; *SI Appendix*, Table S1).

Response to Urbanization: *R. rattus* and *S. muelleri*. To further explore the impact of urbanization on rodent ecology, we focused on the two most abundant species across the urban–rural gradient, namely, *R. rattus* and *S. muelleri*. However, two distinct mitochondrial lineages of the *R. rattus* species complex were identified based on a cytochrome *c* oxidase I (COI) gene PCR assay: *R. rattus* lineage R3 and *R. rattus* lineage *tanezumi*. Multiple lines of evidence now suggest that these lineages should both be considered *R. rattus*; therefore, they were combined for all further analyses unless otherwise specified (14, 21, 22).

Across the urban–rural gradient, 73% of all sites contained *R. rattus*, while 43% of sites contained *S. muelleri*. The proportion of sites with *R. rattus* increased with increasing urbanization, whereas *S. muelleri* was most common at sites in the developing location (Fig. 2). The proportion of sites at which both species were collected also increased across the gradient, with significantly more shared sites in the urban and developing locations (47.4% and 41.7% of sites shared, respectively) than in the rural location (16.9%; $X^2 = 9.17$, $P = 0.01$). Notably, we found that location alone was not a strong predictor of either *R. rattus* or *S. muelleri* presence at a site (Table 1). As a result, we ordinated our study sites using nonmetric multidimensional scaling (NMDS) and nine environmental variables, which resolved highly orthogonal principal axes in three dimensions. These three axes explained 86.3% of the variation in our data, and the stress of the final solution was less than 99.3% ($P < 0.004$). Strikingly, our study sites did not cluster strongly by location (rural, developing, urban) across any of the three ordination axes (Fig. 2). This suggests the presence of substantial variation in the fine-scale environmental features present at sites within a location, as well as commonalities in sites between locations.

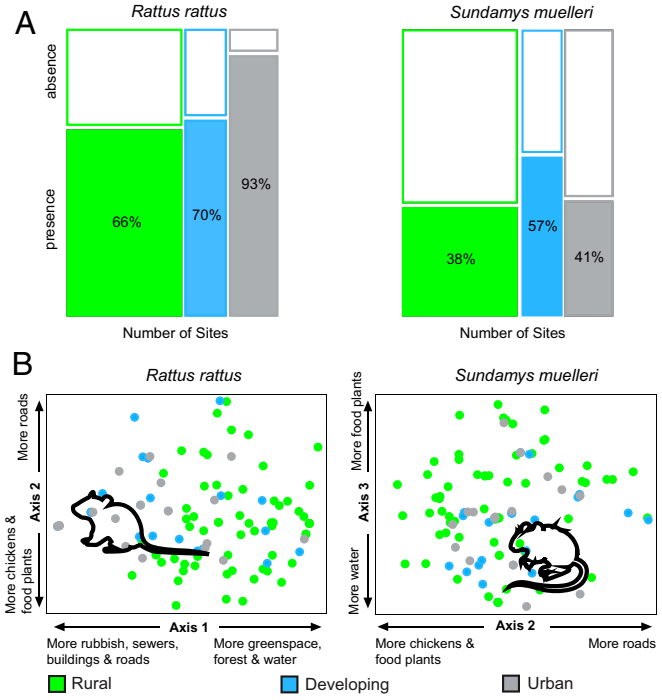

Fig. 2. Distribution of *R. rattus* and S. *muelleri* across the urban–rural gradient and the environmental factors that best predict their presence. (*A*) The proportion of sites positive for each species is shown on the vertical axis with the number of sites sampled represented on the horizontal axis. Green represents the rural location, blue the developing location, and gray the urban location. (*B*) The distribution of rural (green circles), developing (blue circles), and urban (gray circles) sites ($n = 115$) is shown according to the three principal axes ordinated by NMDS. The environmental features that are significantly associated with each principal axis are presented on the x- and y-axes, and the position of the rodent symbol indicates the predicted relationship between each rodent species and these environmental features.

Generalized linear models (GLMs) were constructed to explore the association between *R. rattus* and *S. muelleri* and the three ordination axes, which revealed a highly significant relationship between the presence of *R. rattus* and axes 1 and 2, and a significant relationship between the presence of *S. muelleri* and axes 2 and 3 (Table 1 and Fig. 2). Axis 1 explained the majority of the variation in our environmental data ($R^2 = 0.587$) and distributed the sites based on a gradient of human infrastructure, while axis 2 ($R^2 = 0.157$) reflected a gradient of small-scale domestic agriculture (Table 2 and Fig. 2). Axis 3 explained the least amount of data variation ($R^2 = 0.119$) and placed the sites on a gradient primarily driven by the presence of natural waterbodies. Taken together, these results suggest that *R. rattus* was significantly more likely to be found at sites with built infrastructure, including buildings, roads, rubbish, and sewers, while *S. muelleri* was more likely to be found at green sites with flowing water and without food plants or chickens (Fig. 2). However, due to the smaller number of *S. muelleri* sampled (and smaller number of sites with *S. muelleri* present), we have less statistical confidence in our ability to predict the presence of this species based on the three ordination axes (Table 1).

Finally, we examined the body mass index (BMI) of adult *R. rattus* and *S. muelleri* at each location as a proxy for the body condition of individuals. Male BMIs were significantly different from female BMIs for both *R. rattus* (unpaired $t = 2.89$, df = 353, $P = 0.004$) and *S. muelleri* (unpaired $t = 6.00$, df = 279, $P < 0.0001$); therefore, each sex was analyzed separately (Fig. 3). Overall, the BMI of individuals from both species varied significantly by location ($P < 0.002$) for all sex and species combinations. Pairwise comparisons revealed that male and female *R. rattus* and *S. muelleri* from the urban location were significantly heavier than those from the rural location (Bonferroni-adjusted $P < 0.0167$). Similarly, male *S. muelleri* and both male and female *R. rattus* from developing areas were significantly heavier than individuals from the rural location, while female *S. muelleri* from the urban location were significantly heavier than those from the developing location (Bonferroni-adjusted $P < 0.0167$).

Ectoparasites and the Urban–Rural Gradient. Of the 815 animals from which ectoparasite collection was attempted, only 105 rodents (12.8%) were free of any ectoparasite. Mites (Mesostigmata) were most commonly identified, followed by ticks (Ixodida), lice (Phthiraptera), and fleas (Siphonaptera) (*SI Appendix*, Table S2). Coinfestations were routinely identified, with 188 animals (23.1%) infested by at least two groups of ectoparasite. Of the two most abundant rodent species, mites were significantly more likely to be found on *S. muelleri* (Fisher exact test, $P < 0.001$), where they were also more abundant on each individual (personal observation, *SI Appendix*). The following three genera of hard ticks (family *Ixodidae*) were

Table 1. Results of binomial GLMs that describe the presence of *R. rattus* and *S. muelleri* across the urban–rural gradient

Model		Estimate (SE)	Z-value	Pr(>|z|)	χ^2 (P)
R. rattus: location	Intercept	0.67 (0.26)	2.56	0.011*	
	Developing	0.16 (0.52)	0.30	0.77	
	Urban	1.86 (0.78)	2.38	0.017*	
R. rattus: ordination	Intercept	1.44 (0.30)	4.79	$1.7e^{-6}$**	0.84 (0.66)
	Axis 1	−1.60 (0.44)	−3.62	$3.0e^{-4}$**	
	Axis 2	−1.70 (0.55)	−3.08	0.0021*	
	Axis 3	1.00 (0.51)	2.00	0.05*	
S. muelleri: location	Intercept	−0.47 (0.25)	−1.84	0.065	
	Developing	0.73 (0.49)	1.49	0.137	
	Urban	0.10 (0.47)	0.20	0.84	
S. muelleri: ordination	Intercept	−0.33 (0.21)	−1.57	0.12	1.02 (0.60)
	Axis 1	0.62 (0.29)	2.16	0.031*	
	Axis 2	1.49 (0.51)	2.95	0.0032*	
	Axis 3	−1.42 (0.48)	−2.90	0.0034*	

Values are shown for 1) estimated regression coefficients (Estimate) with SE, 2) Z-values, and 3) *P* values (Pr(>|z|)). Significance at $\alpha = 0.05$ is indicated by *; significance at $\alpha = 0.001$ is indicated by **. The value for the Hosmer-Lemeshow χ^2 and associated *P* value (P) is also given for each best model.

Table 2. Pearson correlation coefficients of environmental variables with ordination axes

Variable	Ordination axis 1	Ordination axis 2	Ordination axis 3
Fraction buildings	−0.715***	−0.240	0.115
Chickens	0.362*	−0.299*	0.274
Food-plants	0.340*	−0.390**	0.441**
Greenspace type	0.549***	0.093	0.088
Fraction greenspace	0.764***	−0.100	0.227
Roads	−0.511***	0.379**	−0.020
Rubbish	−0.807***	−0.309*	0.018
Sewers	−0.738***	−0.152	0.294*
Waterbody	0.553***	−0.522***	−0.647***

Significance is indicated as follows: *$P < 0.005$; **$P < 0.0001$; ***$P < 0.00001$.

identified: *Amblyomma* spp., *Haemaphysalis* spp., and *Ixodes* spp. All three life stages (i.e., larvae, nymphs, adults) of *Amblyomma* ticks were present only on *R. rattus*, whereas all three life stages of *Ixodes* ticks were collected only from *S. muelleri* (*SI Appendix*, Table S3). All tick genera were more strongly associated with *S. muelleri* than *R. rattus*, with significantly more *S. muelleri* individuals infested (Fisher exact test, $P = 0.00013$). This pattern was also observed when each tick life stage was examined separately; *S. muelleri* was significantly more likely to be infested with *Haemaphysalis* and *Amblyomma* larvae, all three genera of nymphs (Fisher exact test, $P < 0.001$), and *Ixodes* adults. Although *S. muelleri* was also more commonly parasitized by *Ixodes* larvae than *R. rattus*, this result was not significant (Fisher exact test, $P = 0.10$). Finally, individual *S. muelleri* were the most heavily parasitized species in this study; of rodents with at least 1 tick present ($n = 144$), 70% belonged to *S. muelleri*, as did 14 of the 15 individuals with more than 10 ticks.

Due to the abundance of ticks and their association with important zoonotic pathogens, we further examined their distribution across the urban–rural gradient (Fig. 4). We found a strong association between urban environments and the presence of *Amblyomma* ticks ($\chi^2 = 77.7$, df = 2, $P < 0.00001$), whereas *Ixodes* ticks were never encountered on a rodent from an urban site ($\chi^2 = 22.89$, df = 2, $P = 0.00001$). *Haemaphysalis* ticks showed divergent patterns across the urban–rural gradient depending on the host; *S. muelleri* individuals in urban environments were more commonly infested than individuals in the other locations (Fisher's exact test, $P < 0.00001$), whereas rural *R. rattus* were much more likely to be infested than individuals from either developing (Fisher's exact test, $P = 0.0002$) or urban locations (Fisher's exact test, $P < 0.00001$) (Fig. 4). Bipartite networks constructed to examine the interactions between rodents and ticks at each location (rural, developing, urban) across the gradient revealed that all three of the rodent–tick networks were significantly modular, indicating that subsets of rodents and parasites interact more strongly among themselves than with other subsets (modules). Networks from the rural and developing locations were similar in structure, with four modules in each and similar degrees of connectedness (Table 3). In contrast, the urban rodent–tick network was less fragmented, with only two modules and a higher degree of connectedness across the network. This is consistent with the hypothesis of increased sharing of ticks between individuals in urban areas. An analysis of the projected unipartite networks for each location further supported this hypothesis—the edge density of the urban rodent–tick network was significantly higher than the edge density of the rural and developing networks, which were highly similar to each other (Table 3). Examination of the top 20 most central nodes (i.e., those with the highest eigenvalues) in the projected unipartite networks also revealed similarities between the rural and developing locations, with a large number of collection sites and rodent species contributing to tick transmission at each location (*SI Appendix*, Table S4). In contrast, all 20 of the most central nodes in the urban network were occupied by individuals belonging to *S. muelleri* collected from only five sites, indicating their importance as hosts in the urban environment.

Microbes and the Urban–Rural Gradient. We tested 316 rodents for potential zoonotic pathogens and found evidence for the presence of coronaviruses (GenBank accession numbers MH107323 to MH107330), mammarenaviruses (MG999643), orthohantaviruses (MH236804 to MH236810), orthohepeviruses (MH236817 to MH236830), and paramyxoviruses (MH236811 to MH236816), as well as *Bartonella* spp. (MG807665 to MG807845), *Leptospira* spp (9), and *Toxoplasma gondii*, in at least one individual (*SI Appendix*, Table S5). No animals were positive for alphaviruses, flaviviruses, enteroviruses, *Rickettsia* spp., or *Yersinia pestis* by the assays used in this study. While a

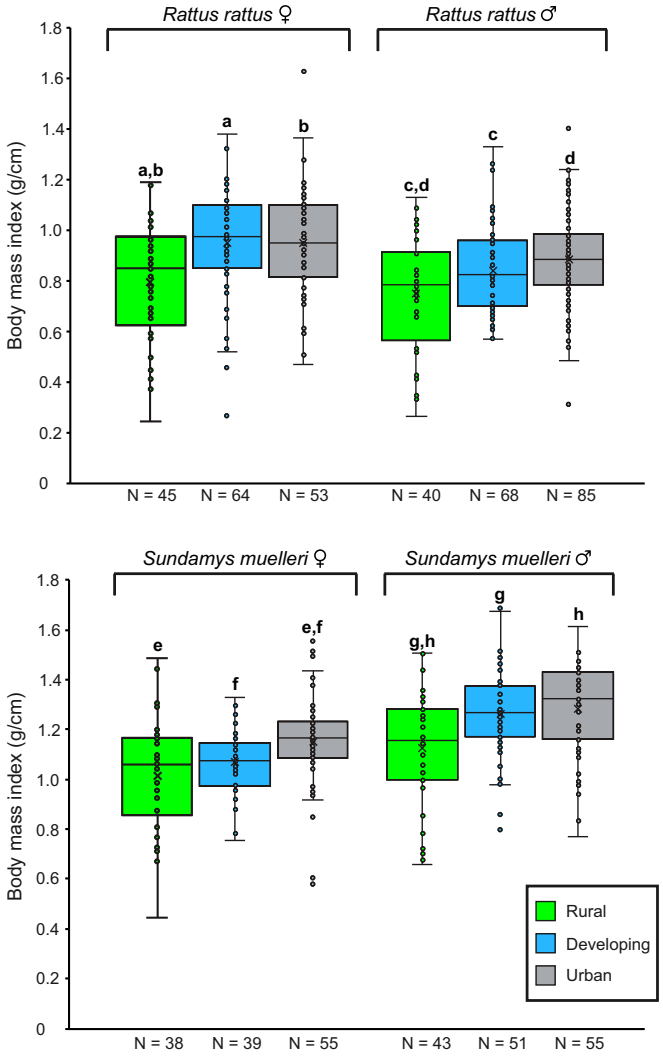

Fig. 3. Box plots showing the adult body mass indices (BMIs) of male (♂) and female (♀) *R. rattus* and *S. muelleri* across the urban–rural gradient. Within each location, the BMI of each rodent is indicated by a circle and colored by location, namely, rural (green), developing (blue), and urban (gray). For each comparison, significant differences in BMI between locations are indicate by lowercase letters.

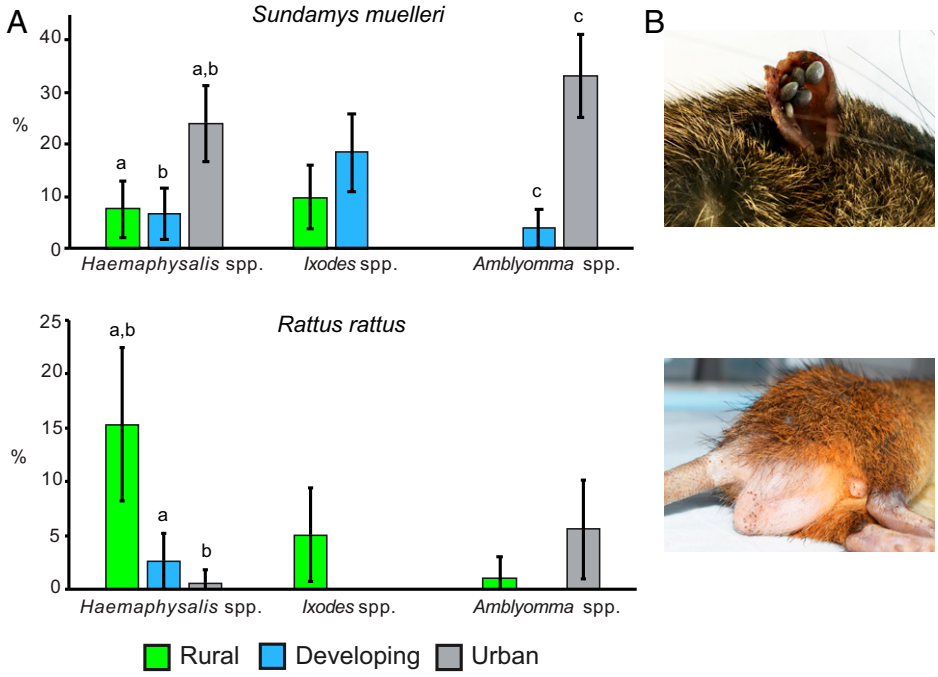

Fig. 4. Association between *S. muelleri*, *R. rattus*, and ticks across the urban–rural gradient. (*A*) Proportion (%) of rodents with ticks identified as either *Haemaphysalis* spp., *Ixodes* spp., or *Amblyomma* spp. across the urban–rural gradient. The rural location is indicated by green bars, developing by blue, and urban by gray bars. Significant differences in the proportion of individuals with ticks at each location is indicated by lowercase letters. (*B*) Photographs showing ticks in situ on *S. muelleri* from the urban location.

single, previously described species was recovered from the mammarenavirus (*Wenzhou mammarenavirus* [WENV]), orthohantavirus (Serang virus [SERV]; a variant of *Thailand orthohantavirus*), orthohepevirus (*Orthohepevirus C* genotype 1, HEV-C1), and *T. gondii* PCR assays, multiple species or genotypes were detected by the remaining assays. Phylogenetic analysis of the coronavirus sequences revealed the presence of two clades belonging to a single *Betacoronavirus* species most closely related to several *Betacoronavirus* lineage A isolates from Southeast Asian rodents (subgenus *Embecovirus*), as well as human coronavirus HKU1 (*SI Appendix*, Fig. S2). No clustering by host species was observed within this group. In contrast, phylogenetic analysis of the paramyxovirus sequences revealed two distinct species (*SI Appendix*, Fig. S3). The first was associated with *S. muelleri* (putatively named Rodent paramyxovirus SM) and appears distantly related to the *Morbillivirus* genus, along with viruses sequenced from *R. rattus* in Nepal (PREDICT_PMV-55/PR0225) and *Arvicanthis* sp. in Zambia (Rodent Paramyxovirus LR11-142). The second paramyxovirus (putatively named Rodent paramyxovirus R3) may represent a new species of *Jeilongvirus*, along with PREDICT_PMV-58/VN13M0007 from *Rattus* sp. in Vietnam and was detected in the kidneys of three *R. rattus* lineage R3 individuals. Multiple species of *Bartonella* and *Leptospira* were also identified, and the results of these assays have been reported in detail previously (9, 23). In brief, *Bartonella* spp. was the most commonly detected pathogen in this study, with 57% of rodents infected from five species. Several human pathogens were identified, including *Bartonella elizabethae* and *Bartonella tribocorum* (both identified in urban *R. rattus*) and *Bartonella rattimassiliensis* (present in *R. rattus* across the urban–rural gradient). We also identified the two most significant causes of leptospirosis globally (*Leptospira interrogans* and *Leptospira borgpetersenii*) in 32% of rodents, combined. Overall, the prevalence of infection was low for most of the microbes surveyed here; however, both the diversity and prevalence of rodent-borne microbes identified in this study increased with increasing urbanization, and some pathogens (SERV, WENV), were only present at urban sites (Fig. 5).

An examination of the bipartite networks constructed to examine interactions between rodents and microbes at each location ($n = 3$) across the urban–rural gradient revealed a weaker relationship between urbanization and community structure than was observed for the rodent–tick networks (Table 3). Networks from the developing and urban locations were similar in structure (eight modules each), indicating a higher degree of connectedness, whereas the rural–microbe

Table 3. Metrics associated with bipartite networks describing the interactions between rodents and ticks or rodents and microbes at each location across the urban–rural gradient

Network	Location	Connect	Modularity (N)	Temp	ED (95% CI)
Rodent:tick	Rural	0.20	0.68 (4)	18.7	0.32 (0.35–0.41)
	Developing	0.18	0.66 (4)	25.9	0.25 (0.21–0.26)
	Urban	0.31	0.56 (2)	19.5	0.52 (0.48–0.58)
Rodent:microbe	Rural	0.15	0.71 (5)	6.3	0.24 (0.21–0.29)
	Developing	0.12	0.59 (8)	7.3	0.32 (0.29–0.36)
	Urban	0.11	0.51 (8)	13.5	0.37 (0.29–0.51)

The location, connectedness (connect), modularity (number of modules), nestedness temperature (temp), and edge density (ED) with 95% confidence intervals are shown.

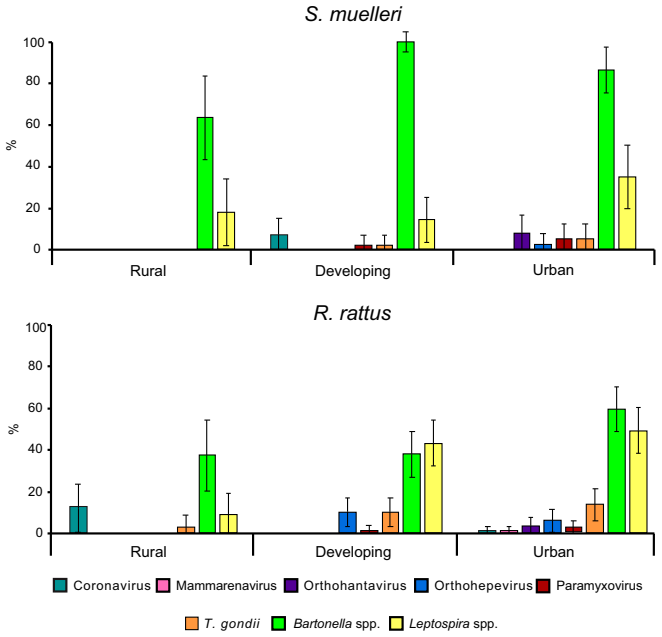

Fig. 5. Proportion (%) of individuals belonging to *S. muelleri* and *R. rattus* infected by each pathogen at rural, developing, and urban locations across the urban–rural gradient. Pathogens were identified using consensus PCR assays and confirmed by Sanger sequencing.

network was more strongly partitioned. As with the rodent–tick system, we found an increasing edge density in the projected unipartite networks with increasing urbanization, although this result was not statistically significant (Table 3). Examination of the top 20 most central nodes in the unipartite networks also revealed similarities between the developing and urban locations that appear to be driven by the importance of *R. rattus* as a microbial reservoir in these locations (65% of the top 20 central nodes were occupied by *R. rattus* in the developing and urban networks) (*SI Appendix*, Table S4). In contrast, 63% of the most central nodes in the rural network were occupied by *S. muelleri*, with *R. rattus* occupying only one-quarter of central nodes.

Discussion

In this study, we explored the impact of urbanization on zoonotic disease risk by characterizing changes in the ecology of animal reservoirs (rodents), ectoparasite vectors (ticks), and potential pathogens across a gradient of urbanization in Malaysian Borneo. The goal of this work was to begin to identify habitats, species, or behaviors that may alter the risk of zoonotic disease emergence in tropical cities and provide a foundation for the development of risk reduction measures. Predictably, we found that rodent species diversity decreased with increasing urbanization, leaving an urban species assemblage that was dominated by two species—*R. rattus* (an urban exploiter) and *S. muelleri* (an urban adapter). Although these species were occasionally found in the same microhabitats across the urban–rural gradient, habitat partitioning was more common, with *R. rattus* more frequently found within the built environment and *S. muelleri* strongly associated with the presence of green space. Spatial segregation between human commensals and native species has been observed previously and may be a common feature of cities (4, 5). As a result, the location, frequency, and intensity of contact between people and urban exploiters/adapters is likely to differ across the urban landscape, as are the implications for zoonotic disease risk.

We found that *R. rattus* was significantly more likely to be present at urban sites than developing or rural sites and strongly preferred those with human infrastructure. Accordingly, we found that rural animals had significantly lower BMIs than those trapped in locations with higher anthropogenic influence, which may be the result of decreased resource provisioning or reduced lifespan in rural areas. This is consistent with the hypothesis that *R. rattus* benefits from a close association with people and the accompanying resources and infrastructure we provide (3). Although *R. rattus* was strongly associated with the urban location in this study, we found that classifying sites into rural, developing, or urban categories alone was not a good predictor of the presence of *R. rattus* (or *S. muelleri*) across the landscape. This suggests that features of the local environment (i.e., at site level) may be more important predictors of species diversity and abundance than landscape context. Indeed, we found evidence that reclassifying our sites using fine-scale environmental variables better predicted species presence than location alone, especially for *R. rattus* (Fig. 2). Specifically, sites with sewers, rubbish, buildings, and roads were highly correlated with the presence of *R. rattus* across the urban–rural gradient, while extensive green space and rivers were associated with an absence of this species. Although these habitat preferences likely translate to a reduced risk from tick-borne pathogens, our results also suggest that there may be an elevated risk from pathogens capable of environmental transmission (e.g., *Leptospira* spp., *T. gondii*, HEV-C1) in habitats where *R. rattus* and other synanthropic species thrive. This indicates that some features of the built environment may support pathogen persistence in urban areas, including open sewers, markets, and colocated commercial and residential activity (9).

For example, we found that all *Leptospira* species (and *L. borgpetersenii* in particular) were statistically more likely to be present at sites with higher anthropogenic activity across the urban–rural gradient and were most frequently detected in *R. rattus* (9). This finding was unexpected, as leptospirosis is predominantly considered a rural disease and recent Malaysian outbreaks have been associated with rural recreational activities (12, 24). However, several features of urban Kuching may support the environmental transmission of *Leptospira* spp., thereby contributing to the persistence of this pathogen in the city. These include open sewers, high annual rainfalls, and a warm, humid climate (9). Similarly, interactions between people, animals, and the built environment appear to be driving the relatively high prevalence of *T. gondii* in urban *R. rattus* detected in this study. *T. gondii* was the only pathogen surveyed whose prevalence increased consistently with urbanization across the gradient, and this trend may be driven by the importance of domestic cats as the definitive hosts of *T. gondii*. Cats were strongly associated with human habitation across our study sites and were much more frequent in the urban location, as was *R. rattus* (25). Rodents act as the primary source of infection for cats, and in this study, *T. gondii* was almost entirely associated with the presence of *R. rattus*. However, human infection occurs through the ingestion of food, water, or soil contaminated with cat feces and the oocysts they contain, which can remain infectious in the environment for up to 1 y in humid conditions (26). Together, these factors may help explain the association between *R. rattus* and *T. gondii* in the built environment, where *R. rattus* was frequently captured in and around sewer systems that are conducive to water-borne transmission (27). Human infections with *T. gondii* are frequent in Malaysia, with a seroprevalence in healthy people that ranges from 14 to 30% and varies with ethnicity, sex, age, and socioeconomic status (28).

This suggests that zoonotic transmission of *T. gondii* is relatively frequent in this area and may be driven by high densities of cats, rodents, and people all within an environment that is well-suited to transmission.

The large, dense populations of *R. rattus* and other urban exploiters/adapters in the built environment may also support the circulation of pathogens that exhibit density-dependent transmission (29). For example, two pathogens were found only in the urban location—SERV (an orthohantavirus) and WENV (a mammarenavirus). Rodents are the primary reservoir hosts for both orthohantaviruses and mammarenaviruses, and transmission between rodents can occur through both vertical and horizontal routes (30, 31). In this study, SERV was detected in *R. rattus* from a single site (a large market in the city center—also the only site where WENV was detected) and in *S. muelleri* from three vegetated sites, of which two also contained *R. rattus.* It is unclear why these rodent-borne viruses were not detected in developing or rural sites; however, several factors may explain their absence, including the following: 1) a lower population density of competent hosts in the rural and developing locations, which can reduce transmission below the level necessary for persistence; 2) the presence of less-competent hosts in more diverse rodent communities, which may reduce intraspecific interactions and horizontal transmission (i.e., the dilution effect); 3) recent introductions of infected rodents through shipping or other routes of importation into Kuching's city center; or 4) an artifact of our relatively small sample size that may have inadvertently excluded microhabitats more conducive to viral transmission (32–34). It is therefore possible that these (and other) zoonotic pathogens are circulating in rodents at low frequency across the landscape and yet were not detected here. Notably, both SERV and WENV have recently been described in Southeast Asia, and as with many other rodent-borne zoonoses, little is known about the epidemiology or public health risks associated with these viruses (35, 36). However, the presence of SERV at multiple urban sites with differing microhabitats, and in at least two reservoir species, suggests that this virus is endemic to the local area and may pose a risk to people where contact with rodents is high. In contrast, our singular detection of WENV from *R. rattus* in Kuching's city center suggests that the risk of zoonotic transmission of this virus is likely minimal. As many rodent-borne pathogens have a similar clinical presentation and are frequently misdiagnosed, the public health significance of these viruses may be underestimated (7, 36). As a result, targeted human serosurveys will be necessary to determine if the pathogens identified in urban rodents in this study are associated with health risks to people.

Unlike *R. rattus*, *S. muelleri* was most prevalent in the developing location, which may suggest a preference for disturbed or fragmented landscapes that provide access to both natural habitat and the increased resources associated with human habitation. *S. muelleri* has previously been observed occupying patches of remnant vegetation, parks, and fallow land in urban and developing areas of Borneo, where it has been found at relatively high abundances (22, 37–39). Together, these data strongly suggest that *S. muelleri* should be considered an urban adaptor species in Southeast Asia. We were less successful in predicting the presence of *S. muelleri* than *R. rattus* based on fine-scale environmental variables; however, like many urban adapter species, *S. muelleri* demonstrated a clear preference for vegetated sites in urban areas, where individuals were both plentiful and heavily infested with ticks (4). Interestingly, the abundance and distribution of ticks also varied significantly across the urban–rural gradient and by genus, reiterating the pattern we observed with rodents (Fig. 4). Urbanization can impact the abundance and distribution of ticks by altering the availability of suitable hosts or by changing the external environment used by ticks when off-host, where they may spend up to 99% of their lifespan (40). Moreover, for a population of ticks to be maintained at a site over time, suitable hosts for all life stages must be present in an environment that also supports off-host behaviors like questing (40, 41). In this study, we found that rodents, and *S. muelleri* in particular, appear to be suitable hosts for both the larval and nymph stages of all three genera of ticks across the landscape; however, adult ticks were found relatively infrequently, particularly in the urban location.

Ticks are capable of transmitting a greater diversity of pathogens than other ectoparasites, and all three genera of tick identified in this study have been associated with zoonotic pathogens, including *Rickettsia* spp., *Bartonella* spp., *Borrelia* spp., and tick-borne encephalitis virus (40, 42). Ticks can become infected with a pathogen during blood feeding at any life stage; however, ticks that feed on the same host species across multiple life stages are more likely to become infected and to transmit this infection during subsequent feedings (43). In this context, the dense urban populations of *S. muelleri* that were heavily infested with multiple life stages of ticks may pose a particular risk to people should a zoonotic pathogen be present in the population. Although we did not identify any known tick-borne pathogens in this study, the use of select consensus PCR assays for pathogen detection may have restricted our ability to identify important zoonoses already circulating in this area (e.g., *Borrelia* spp.). However, a metagenomics-based study of the microbes carried by these same ticks (and associated rodents) is currently underway and is expected to reveal a greater diversity of potential pathogens in this region (44). As in many Southeast Asian cities, green spaces across Kuching are heavily utilized by residents for recreational and other activities and are often considered to be clean, healthy urban environments (45). However, the results of this study suggest that these activities may pose underappreciated public health risks akin to those associated with the transmission of Lyme disease in temperate urban green spaces (23, 41). We suggest that effective public health messaging could inform residents of tropical cities about the potential risks posed by ticks in urban green spaces and encourage the use of insect repellants and other preventative measures. These efforts should be supplemented with a targeted pest management plan to reduce rodent density in areas of high abundance and where frequent contact with people is likely. Further studies on the relationships between features of the built environment, the presence of pathogens, and the frequency of zoonotic transmission should be prioritized to further understand how the structure and use of cities influence zoonotic disease risk.

In summary, we propose a model that indicates that the impact of urbanization on zoonotic disease risk is likely to be a result of interactions between the response of animals, ectoparasite vectors, pathogens, the surrounding environment, and human behavior (Fig. 6). We suggest that the process of urbanization can influence zoonotic disease risk by 1) changing the abundance and distribution of available hosts and/or vectors, impacting pathogen persistence; 2) changing the community composition of hosts/vectors, altering inter- and intraspecific interactions as well as competence; 3) changing the behavior, movement patterns, and immune status of hosts, altering disease susceptibility and transmission; 4) changing the structure and utility of the environment in a manner that influences

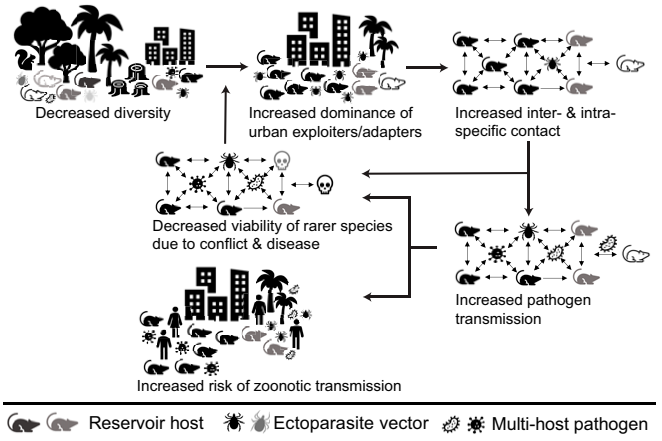

Fig. 6. Proposed model of the impacts of urbanization on the transmission of multihost pathogens and zoonotic disease risk. Urbanization results in decreased host diversity in cities and habitat partitioning of the remaining species. This leads to increased dominance of urban exploiters (solid-black rodents) and urban adapters (solid-gray rodents) in urban environments, as well as the ectoparasites that favor these hosts/habitats (black ticks). As inter- and intraspecific contact between urban exploiters, urban adapters, and their ectoparasites increases so does multihost pathogen (bacteria and virus shapes) transmission, which can lead to a reduced viability of rarer species due to competition, predation, and disease. Together, these factors contribute to a heterogeneous distribution of hosts, vectors, and pathogens in the urban environment, with a higher risk of zoonotic disease transmission arising in habitats where frequent contact between hosts, vectors, pathogens, and people are most likely to occur.

transmission (e.g., increased flooding, poor sanitation, high-density housing); and 5) changing the distribution or transmission dynamics of a pathogen directly. How these processes interact to influence human disease risk in cities will vary by geographic location and by the scale and speed of land-use change, among other factors.

Future research efforts should build upon the whole-of-system approach we demonstrate in this paper by including epidemiological, serological, or molecular data from people living within the study area. The absence of data from people who represent the end of the zoonotic transmission cycle is the most significant limitation of this study and will be required for the identification of factors associated with genuine zoonotic transmission. It is important to note that the conclusions of this study were derived from data collected from a single Southeast Asia city, which is unlikely to represent all of the pace and patterns of urbanization occurring throughout the region. Given the rapid rate of urbanization in Southeast Asia and other tropical regions of the world, it will be critical to focus future research efforts on additional cities in the tropics, where the public health consequences of a new or re-emerging infectious disease can be severe.

Methods

Ethics Statement. This study was approved by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Australian Animal Health Laboratory's Animal Ethics Committee (#1750) and the Sarawak Forests Department (Permit: NCCD.907.4.4 (JLD.12)-131).

Rodent and Environmental Data Collection. Rodents were collected at three locations representing a gradient of urbanization, including 1) Mount Singai, a rural location with low levels of urbanization, dispersed villages and semidisturbed vegetation; 2) Batu Kawa district, a rapidly developing region on the outskirts of Kuching with moderate levels of urbanization, disturbed vegetation, and both new and old human settlements; and 3) Central Kuching, which is comprised of urban gray/green matrix at the center of Sarawak's largest city and has been urbanized for an extended period (Fig. 1). A detailed description of each location is provided in Blasdell et al. (9).

Rodent trapping within each location was conducted at multiple sites, which were opportunistically selected based on accessibility. Every attempt was made to include sites that represented both the built environment (gray space) and the natural environment (green space) at each location. Sites were defined as a circle of 110-m radius centered at the point where global positioning system (GPS) coordinates were taken (Fig. 1). The 110-m radius was chosen to correspond with the approximate home range of *R. rattus* estimated under similar environmental conditions, as this was the only study species for which suitable home range data were available (46). Sites were categorized based on the dominant land-use type observed within the complete circle (i.e., commercial, forest, residential/village, scrub, and mixed commercial/residential), and if a site was on (or next to) an ecotone, this was also noted. A number of site-specific environmental variables were measured or estimated by ground truthing at the time of rodent collection, including the presence of 1) waterbodies (e.g., river, stream, swamp, pond), 2) sewers, 3) livestock (e.g., chickens, pigs, other), 4) food plants (e.g., banana, corn, sugar), 5) rubbish, 6) roads, 7) buildings and building condition, 8) commercial food storage/service/preparation areas, and 9) green space (*SI Appendix*, Table S6).

Rodent trapping was conducted across five time intervals that bracketed the wet season: 1) September/October 2015, 2) March/April 2016, 3) September/October 2016, 4) March/April 2017, and 5) September/October 2017. Rather than equalizing the trapping effort at each location, we attempted to collect a minimum of 50 rodents per location per time. This resulted in sampling from a total of 27 urban, 23 developing, and 65 rural sites ($n = 115$ total) across the study period, due to a lower number of animals captured per site in the rural area (Fig. 1). Trapping was conducted at each site for between one and seven nights using locally made wire mesh traps (~30 cm × 14 cm × 14 cm), baited daily with meat and fruit, and placed at intervals of >1.5 m. Traps were opened between 4 PM and 6 PM daily and collected/closed between 6 AM and 8 AM the following morning. At the time of collection, rodents were morphologically assigned a species identification and either immediately released ($n = 47$) or euthanized by overanesthetization in isoflurane followed by bilateral thoracotomy ($n = 815$). For those animals that were euthanized, carcasses were fumigated using ethyl acetate for ~5 min to kill ectoparasites and combed with a fine-toothed flea comb. Ectoparasites were sorted visually by category (i.e., mites, fleas, lice, ticks) and placed either in ethyl alcohol for further identification or frozen directly on dry ice. Ticks were identified using locally relevant taxonomic keys and texts (38, 47–50). We were only able to identify ticks to the genus level due to a lack of larval and nymphal keys, as these life stages comprised the vast majority of our collection. Lice (Phthiraptera) and fleas (Siphonaptera) were identified to order, and mites were identified to the subclass Acari. The weight, sex, and reproductive status of each animal was recorded, and tissue samples (blood, ear, lung, kidney, liver, spleen) were aseptically collected and frozen directly on dry ice prior to storage at −80 °C.

Landcover Estimates. Spatial data that could be used to quantify landcover, and other measures of urbanization were limited for the study area. Those datasets that were available, such as the Global Road Inventory Project (GRIP) (https://datacatalog.worldbank.org/dataset/grip-global-roads-inventory-project-2018), the Global Urban Landcover dataset (ftp://115.239.252.28/GlobalUrbanLand/), and the WorldPop demographic dataset (https://www.worldpop.org/), were global in extent and did not have a spatial resolution that was appropriate for the 110-m radius we were using to characterize the sites. This limited availability of spatial data is a common issue for many locations in the Global South. Therefore, site-specific landcover estimates were calculated using normalized difference vegetation index (NDVI) values generated from LANDSAT 8 data collected on September 14, 2017 (https://earthexplorer.usgs.gov/). We used the Semi-Automatic Classification Plugin v6.2.9 in QGIS v3.2.3 to transform the digital numbers for LANDSAT 8 data into reflectance values (51, 52). These values were used to calculate the NDVI for each site using the QGIS Raster Calculator tool and the equation: NDVI = (Band 5 – Band 4)/(Band 5 + Band 4), where Band 4 = red and Band 5 = near-infrared. Pixels with NDVI values of −1.000 to 0.150 were considered water, 0.151 to 0.700 were considered urban (gray), and 0.701 to 1.000 were considered green. For downstream analyses, we calculated 1) NDVI_mean as an estimate of the average proportion of green

landcover at each site; 2) NDVI_stdev as an estimate of landcover variability across a site; 3) distance_to_urban, the distance from the center of each site to the nearest urban pixel (an indicator of access to resources associated with urban landcover); and 4) distance_to_water, the distance from the center of each site to the nearest water pixel.

Molecular Analyses: Rodent Species Confirmation and Microbe Detection. DNA and RNA were extracted from ∼30 mg of homogenized tissue using the AllPrep DNA/RNA mini kit (Qiagen) as per the manufacturer's instructions. Nucleic acid quality and concentration for each sample were assessed using the NanoDrop-1000 spectrophotometer (Thermo Scientific), and extractions were repeated for samples with a 260/280 of <1.8 or that yielded <50 ng/μL of nucleic acid. cDNA synthesis was performed on each RNA extraction using SuperScript III (Invitrogen), 50 ng of random primers, and 50 to 200 ng of template RNA, following the manufacturer's protocol. The tentative species assignment given to each animal at the time of collection was confirmed by sequencing the product of a PCR assay using primers BatL5310 and R6036R to amplify ∼750 bp of the COI gene (53). This was followed by BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to assess sequence similarity and assign a species identification.

Samples from all animals collected during the first year of sampling (n = 316) were screened using molecular methods to identify common rodent-borne microbes, of which many are zoonotic pathogens. These were comprised of individuals belonging to the species *Maxomys whiteheadi* (n = 12), *Maxomys ochraceiventer* (n = 2), *Niviventer cremoriventer* (n = 14), *Niviventer* sp. (n = 1), *Rattus rattus* lineage R3 (n = 165), *R. rattus* lineage *tanezumi* (n = 11), *R. tiomanicus* (n = 8), *Rattus exulans* (n = 3), and *S. muelleri* (n = 100), which were all collected between September 2015 and April 2016. Previously published PCR assays were used to screen appropriate tissue and nucleic acid types for selected viruses: alphaviruses, flaviviruses, enteroviruses, mammarenaviruses, coronaviruses, orthohantaviruses, orthohepeviruses, and paramyxoviruses; bacteria: *Bartonella* spp., *Leptospira* spp., *Rickettsia* spp., and *Yersinia pestis*; and the intracellular parasite *T. gondii* (*SI Appendix, Supplemental Methods* for details of tissues and assays). PCR products with appropriately sized amplicons in any assay were subjected to Sanger sequencing, followed by BLAST analysis to assess sequence similarity. Multiple sequence alignments and phylogenetic trees were constructed when further analysis was required and were performed using MUSCLE v3.8 and PhyML v3.1, respectively (54, 55).

Statistical Analyses: Rodent Species Richness. Rodent species richness was estimated using the first-order jackknife (Jack1) and bootstrap method estimators in the package vegan, implemented in R (56, 57). Rarefaction curves were used to estimate the number of rodent species present (as a function of the number of sites), for each location. Estimates of β were also calculated in vegan using the Bray–Curtis index. Values of β range from 0 (the community composition at two locations are completely dissimilar) to 1 (community composition at two locations are identical).

Statistical Analysis: Site Classification. Two linear discriminant analyses (LDAs) were used to assess the fit of our a priori assignment of each site into a rural, developing, or urban classification using a subset of environmental variables. The purpose of these analyses was to understand the variability in contemporary land cover within the three areas of the landscape that had been defined a priori based on their urban development histories. The first LDA (LDA_strict) contained only landscape variables that were calculated based on the NDVI produced from the LANDSAT 8 image, namely, NDVI_mean, NDVI_stdev, distance_to_urban, and distance_to_water. This analysis found that classifying sites into rural, developing, or urban locations using only contemporary land cover features produced identical location assignments for 85.2% (98/115) of sites (*SI Appendix*, Fig. S4). Subsequently (LDA_relaxed), we added site-specific variables collected during field sampling that were related to the presence/absence of livestock, sewers, and buildings, which improved the predicted classification to 89.6% (103/115) (*SI Appendix*, Fig. S4). Rural sites were most likely to be consistently classified (60/65 rural sites classified as rural by LDA_strict, 63/65 sites by LDA_relaxed), with the remaining sites classified as developing, most likely because they contained significant anthropogenic influence and incorporated the edge of a village or multi-family dwellings. In contrast, urban sites were slightly less likely to be consistently classified (20/27 urban sites classified as urban by LDA_strict, 21/27 by LDA_relaxed), with the remaining sites classified as developing, as they contained significant proportions of green space (in some cases 100% of the site was covered in vegetation). Similarly, 18/23 (LDA_strict) and 19/23 (LDA_relaxed) developing sites were consistently classified, with the remaining sites assigned to both urban and rural locations. There were no cases where the classification identified an urban site as rural or vice versa. LDAs were performed using the library MASS, implemented in R (58). We tested the assumption of homogeneity of variance across groups using Levene's test for homogeneity of variance using the R package car (59).

Statistical Analysis: Rodent Response to Urbanization. To identify links between rodent presence and the environmental variables measured across the urban–rural gradient, NMDS was first used to ordinate all 115 sites in environmental space using PC-ORD 6.08 (60). This method was chosen as it is well-suited to ecological and environmental data as it is based on ranked distances and therefore does not assume data normality (61). A Sørensen dissimilarity matrix was utilized as the input for this analysis as it retains sensitivity in heterogenous datasets and gives less weight to outliers, as are often observed in ecological datasets. This matrix was calculated based only on those environmental variables for which nonzero values were recorded for at least 50% of sites. These included waterbody (flowing, standing, none); sewers (presence/absence); chickens (presence/absence); food plants (presence/absence); rubbish (new/old/none); roads (presence/absence); dominant green space type (forest, scrub, mixed, garden, none); and the fraction of each site that was green space or buildings, for a total of nine variables. A Monte–Carlo randomization test was applied to determine the stress of the final solution. The resulting ordination axes were correlated with each environmental variable using the Pearson correlation coefficient with a Bonferroni correction for multiple tests to establish a conservative threshold for significance ($P < 0.005$, n = 115 sites). Finally, these ordination axes were used as predictor variables in independent binomial GLMs to examine their influence on rodent presence. GLMs were constructed individually for the two most frequently collected rodent species (*R. rattus* and *S. muelleri*), using the R packages car and ResourceSelection (59, 62). Model fit was assessed using the Hosmer–Lemeshow goodness of fit test. As a comparison, GLMs were also constructed for both *R. rattus* and *S. muelleri* as above, with location (rural, developing, urban) as a predictor. See *SI Appendix, Supplemental Methods* for predictive model evaluations comparing this approach with machine learning approaches.

Statistical Analysis: Rodent Morphometrics. Descriptive statistics (Fisher's exact test, χ^2 test) were used to investigate the relationships between ectoparasite presence/abundance (ticks, lice, mites) and location (rural, developing, urban), as well as associations with rodent species. Exact goodness of fit tests were used to assess differences in trapping success by sex, and one-way ANOVAs followed by paired t tests (with Bonferroni correction for multiple comparisons) were used to examine changes in adult BMIs as a proxy for body condition [calculated as the ratio of body weight (g)/snout-vent length (cm)] between locations (63). No pregnant individuals were included. We focused solely on the two most abundant rodent species for these analyses (*R. rattus* and *S. muelleri*) due to small sample sizes of the remaining species.

Statistical Analysis: Network Analyses. Bipartite networks were constructed to examine the interactions between 1) ticks and rodents and 2) microbes and rodents, at each of the three locations across the urban–rural gradient using the R packages bipartite v2.03 and igraph (64, 65). We tested the hypothesis that there would be increased ectoparasite and microbe sharing between individuals with increasing urbanization in this system. The tick community was described by both genus (*Amblyomma*, *Haemaphysalis*, or *Ixodes*) and life stage (larva, nymph, adult), while microbes and the rodent community were described at the species level. Interactions between rodents and either ticks or microbes were investigated using three qualitative metrics. First, the connectance of each network was calculated as the ratio of observed to possible links between rodents and parasites, which corresponds to the number of species combinations in the system (66). Next, we calculated the modularity of each network to identify subsets (modules) of the network that are more densely connected than other subsets. The modularity of a network quantifies how clustered a network is, with higher values of modularity indicating that subsets of rodents and parasites interact more strongly among themselves (i.e., within a module) than with other subsets (67). Finally, we calculated the nestedness temperature of each network, which provides an

estimate of the orderliness of the network and reflects both species composition and incidence (68). Nestedness temperature values range from 0 to 100, with higher values observed when more abundant parasites interact more frequently with a wider range of hosts. Finally, we transformed each bipartite network into a unipartite network by connecting individual rodents based on shared ticks/microbes using igraph (65). Unipartite networks represent the pattern of relative interactions among individual rodent hosts through the sharing of ectoparasites or microbes. Nonparametric resampling was used to estimate the edge density with confidence intervals of each unipartite network using the R package snowboot (69). If our hypothesis is supported, we would expect to see a structural change in the bipartite and unipartite networks, with a decrease in modularity and an increase in edge density. Finally, eigenvector centrality was calculated from each projected unipartite network to identify individuals, sites, or rodent species occupying central (i.e., well-connected) positions in each network and that may be of importance for the transmission of ticks or microbes at each location.

Data, Materials, and Software Availability. The complete data set associated with this publication is available on Zenodo (DOI: 10.5281/zenodo.5581476) (70). All study data are included in the article and/or *SI Appendix*.

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Author affiliations: [a]Health and Biosecurity Business Unit, Commonwealth Scientific and Industrial Research Organisation, Geelong, VIC, 3220, Australia; [b]Institut des Sciences de l'Evolution de Montpellier, National Center for Scientific Research, Montpellier University, Montpellier, 34090, France; [c]Faculty of Veterinary Technology, Kasetsart University, Bangkok, 10900, Thailand; [d]College of Science and Engineering, James Cook University, Cairns, QLD, 4811, Australia; [e]Data61, Commonwealth Scientific and Industrial Research Organisation, Dutton Park, QLD, 4102, Australia; [f]Department of Medical Entomology, NSWHP-ICPMR, Westmead Hospital, Westmead, 2145, Australia; [g]School of Ecosystem and Forest Sciences, The University of Melbourne, Parkville, VIC, 3050, Australia; [h]The Institute of Health and Community Medicine, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, 94300, Malaysia; and [i]The Australian Institute of Tropical Health and Medicine, James Cook University, Cairns, QLD, 4811, Australia

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