Chapter 2
Genetic Impoverishment
in the Anthropocene: A Tale from Bats

Balaji Chattopadhyay, Kritika M. Garg, Rajasri Ray, Ian H. Mendenhall, and Frank E. Rheindt

Abstract Habitat loss, fragmentation, and anthropogenic climate change are major drivers of biodiversity declines during the ongoing Anthropocene epoch. Understanding the evolutionary trajectories of organisms with diverse life histories in response to these threats can enable us to predict the fate of the extant biota facing accelerated habitat loss and climate change. Genetic data contain vital clues about species diversity and have been widely used to assess the impacts of non-anthropogenic climate change (since the Last Glacial Maximum and during the Holocene) on a range of species. Recent advances in sequencing technologies and analytical approaches have broadened the scope of genetic investigations. They have allowed us to directly test for recent population bottlenecks linked to rapid, anthropogenic environmental change. In this chapter, we discuss the utility of genomic data in identifying evolutionary trajectories of bats in response to climate change and habitat modification. We show that these nocturnal mammals are particularly sensitive to environmental and habitat fluctuations. We also summarise and discuss our recent investigations of an urban population of the lesser short-nosed fruit bat (*Cynopterus brachyotis*) from the island nation of Singapore and assess the

B. Chattopadhyay (✉)
Trivedi School of Biosciences, Ashoka University, Sonipat, Haryana, India

Department of Biology, Ashoka University, Sonipat, Haryana, India
e-mail: balaji.chattopadhyay@ashoka.edu.in

K. M. Garg
Department of Biology, Ashoka University, Sonipat, Haryana, India

Centre for Interdisciplinary Archaeological Research, Ashoka University, Sonipat, India

R. Ray
Centre for Studies in Ethnobiology, Biodiversity and Sustainability (CEiBa),
West Bengal, India

I. H. Mendenhall
Duke-NUS Medical School, Singapore, Singapore

F. E. Rheindt
Department of Biological Sciences, National University of Singapore, Singapore, Singapore

response of this population to rapid urbanisation during the Anthropocene. Comparisons of genetic diversity estimates and evolutionary models through coalescent simulations revealed that this local population had been on a slow decline for centuries but faced a more drastic bottleneck a few decades ago. We also observed an astonishing level of decline in indicators of genetic diversity in the local population over the past century, coinciding with the rapid urbanisation of Singapore. Our observations show that even commonly occurring, synanthropic species of bat (i.e. lives within and appears to benefit from urban environments) have been negatively impacted by rapid urbanisation. Our results also highlight the necessity of assessing the impact of urban green spaces on the evolution and survival of organisms like bats, which often rely on these remnant habitats.

1 Introduction

The Anthropocene is the age of human dominance and urbanisation [1–4], characterised by rapid loss of natural habitats, climate change, and an ongoing biotic extinction crisis of unprecedented proportions [1, 2, 4, 5]. Recent human activities associated with global industrialisation have radically altered ecosystems, with effects approaching the magnitude of natural events such as tectonic movements, glacial cycles, volcanic eruptions, and other major disruptions [4]. The precise start date of the Anthropocene epoch is still debated, but for the purposes of this chapter, we set it at 1945, after which humanity has undergone a period of 'great acceleration' during which human activities increased on an exponential scale [1]. These activities drove an almost unparalleled episode of destruction of natural habitats, sudden rise in global temperature, alteration of the prevalent climate, and the sixth mass extinction in the history of life [2]. The Earth is being stripped of its biodiversity as species are lost at a rate that is magnitudes higher than that of previous mass extinctions, in which comparable losses occurred across tens of millions of years [2, 6]. We are losing species that have not even been discovered or described [7], prompting scientists and governments to prioritise efforts to understand and mitigate damages to wildlife.

One of the key goals of contemporary biological sciences is to understand the vulnerability of species or biotic communities to habitat modification and climate change, not only to conserve and manage biodiversity but also to understand the impact of biodiversity loss on human survival and well-being. Species' vulnerability to future climate change can sometimes be predicted based on their responses to historic climatic alterations and concomitant habitat fluctuations [8, 9]. Quaternary climatic fluctuations caused multiple periods of glacial maxima over the past two

million years that resulted in repeated range shifts for many species globally [8–11]. Significant reduction in global temperatures led to increased glaciation and aridity. This reduced the available habitat for many taxa and forced them into isolated, local refugia. However, sea levels also fell during these periods, connecting previously isolated landmasses (through land bridges) and expanding available habitat for many other taxa [10–12]. These alternating periods of high isolation and high connectivity drove fluctuations in population size and genetic diversity and in some cases increased rates of speciation [8–11, 13, 14]. For example, increasing subdivision and speciation caused by Pleistocene glacial cycles are documented in many bird species that inhabit islands and prefer forested habitats (specialists), while bird species that use a much wider range of habitats (habitat generalists) exhibit lower rates of speciation [13].

Bats are an interesting group with which to study the effects of climate change on population size and genetic diversity. The order Chiroptera is the second largest mammalian order, comprising more than 1400 known species of bat and harbouring an enormous range of cryptic diversity [15–19]. Bats are also keystone species to many ecosystems and serve as excellent bioindicators [20–22]. They are very sensitive to abrupt climatic fluctuations, and heat stress during heatwaves has been linked to mass mortalities in several regions [23–25]. Large, frugivorous species are susceptible to heatwaves, as shown by mass mortalities of fruit bats recorded in Australia during summer heatwaves [25]. The impact of climate change on bats is not always negative as it may enable range expansions to cooler regions as observed in Mexican free-tailed bats (*Tadarida brasiliensis*) [26]. Bats are ubiquitous and often associated with human-modified habitat [20–22], but their evolutionary response to climate change, both historical and current (i.e. human-mediated), is unclear.

To fill this lacuna in the framework of a long-term initiative to understand the effects of climate change on wildlife, we assessed how bats have responded to historic climatic fluctuations and further investigated the links between urbanisation and population endangerment. While studying the long-term effects of Earth's historic climate change, we reconstructed species' evolutionary histories from single genomes (PSMC analysis: pairwise sequentially Markovian coalescent) and palaeohabitats of 11 phylogenetically divergent bat species with a wide range of biological and ecological traits [8]. In that study, we assessed whether changes in palaeohabitats during periods of climatic fluctuations correlate with changes in effective population size. We observed a significant correlation between available palaeohabitat and effective population size during the last glacial period. Frugivores were particularly susceptible to global warming, with their population size dramatically decreasing after the Last Glacial Maximum. Our comparative genomic analysis also indicated that large insectivores generally have a low effective population size and that bat species generally entered the Holocene with low effective population sizes [8]. These observations indicate overall vulnerability of bats to climate change and concomitant habitat fluctuations. They also suggest that a species' biology and ecology play an important role in determining its resilience during future climate change and habitat modifications [8]. Comparative PSMC analyses across vertebrates remain rare in the scientific literature. However, the available studies (e.g. 6 felids and 38 species of birds) reveal similar patterns

of declines in effective population size during the last glacial period for most taxa and especially those that are currently endangered [9, 27].

Studying species' responses to historic climatic fluctuations has highlighted that knowledge of the past can shed light on the effects of more recent climate change. This includes the changes during the past few centuries marked by the industrial revolution in general and specifically during the last few decades that were characterised by ultra-rapid urbanisation, mechanisation, and habitat loss [28]. In many cases, however, the available methods of demographic reconstruction cannot sufficiently capture the signals of these recent effects [28–30]. Rather, a comparison of pre- and post-decline populations can alleviate this limitation and provide resolution of demographic histories during the Anthropocene [28, 30]. In this context, biological collections are a treasure trove. Specimens collected over the last few centuries and preserved in natural history museums worldwide can provide us with genetic samples that reflect a population's status prior to intense urbanisation. This enables a direct comparison with the contemporary (post-urbanisation) population and generates a much deeper resolution into the nature of demographic fluctuations [30]. Next-generation sequencing and bioinformatic pipelines capable of analysing large datasets make it possible to generate and analyse genomic data from degraded museum specimens. This approach has provided insights into historical factors associated with endangerment of natural populations [28, 31, 32]. Armed with these technological advances, scientists hope to leverage species histories to predict their responses to environmental change and their mid- and long-term viability [8, 28].

In old museum specimens, DNA is often heavily degraded due to storage time and chemical damage from historically popular preservatives such as formaldehyde [33]. This has long limited the utility of this valuable resource for genomic analyses. Propitiously, it is now possible to generate large-scale DNA sequencing data even from degraded biological material preserved in museums [33]. For example, a recent study compared DNA from a woolly mammoth (*Mammuthus primigenius*) fossil from about 45,000 years ago, when mammoths were plentiful across the Holarctic region and the effective population size was around 13,000, with those retrieved from a sample dating 4300 years ago from Wrangel Island in the Arctic Ocean, where the population consisted of roughly 300 individuals and represented one of the species' last strongholds prior to extinction [34]. The analysis revealed the accumulation of detrimental mutations in the isolated, island population, consistent with the hypothesis of 'genomic meltdown' prior to extinction.

2   Temporal Genomic Data Reveal Drastic Population Genetic Diversity Decline in a Tropical Fruit Bat

Although bats are one of the most common mammals in anthropogenically altered habitats, the effects of urbanisation on the genetic diversity of bat populations are unclear [8, 22, 28]. As part of our research to understand the sensitivity of bats to Anthropocene habitat alterations, we investigated potential genomic

impoverishment in the Sunda lineage of the lesser short-nosed fruit bat (*Cynopterus brachyotis*) following urbanisation of the island nation of Singapore. This medium-sized, generalist fruit bat is widely distributed across Southeast Asia [35, 36]. It is synanthropic (lives within and appears to benefit from urban environments) and is often observed in cities, towns, and villages, in close proximity to humans. *Cynopterus brachyotis* is broadly sympatric with at least two congeners: the larger Horsfield's fruit bat (*C. horsfieldii*) and a smaller, forest-dwelling species whose taxonomic affinity remains unresolved [36].

Based on its synanthropic tendencies, we predicted that *C. brachyotis* would be resilient to deforestation and would be able to use clusters of urban green space for foraging. The Singapore Strait has largely isolated the population of *C. brachyotis* of Singapore from its nearest neighbours in Peninsular Malaysia since the beginning of the Holocene [37, 38]. Therefore, this study location allowed us to confidently exclude most of the confounding effects of migration and gene flow.

Over the past century, Singapore lost about 95% of its forest cover in parallel massive urbanisation and industrialisation. This caused a considerable loss in biodiversity and an estimated 34–87% loss of species in some taxa experiencing local extinctions [7, 39]. However, careful planning and management have facilitated tree canopy cover over 30% of the island's area, which is high compared with many other large cities (http://senseable.mit.edu/treepedia/cities/singapore).

To understand the impact of urbanisation on a commonly occurring species, we compared the genomic diversity in *C. brachyotis* collected before and after the intensive urbanisation of Singapore. We sampled bats collected in 1931 (pre-industrialisation, pre-urbanisation; $n = 21$) from the Lee Kong Chian Natural History Museum (LKCNHM), Singapore [28], and from the contemporary population, sampled in 2011–2012 ($n = 20$). Genomic diversity of these historic samples was compared to contemporary samples collected in 2011–2012 ($n = 20$). We isolated DNA in dedicated ancient DNA facilities designed for work with historic samples. We targeted ~1.5 Mb of the *C. brachyotis* genome through sequence capture methods, which are highly effective in comparing similar regions of the genome among samples [28, 40]. We designed our own sequence capture panel to target 1184 loci distributed across the genome (see Fig. 2.1 for target locus design). These loci were
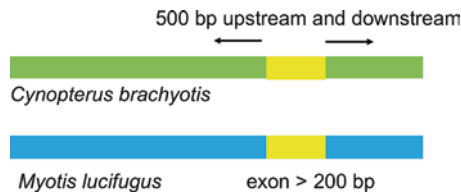

Fig. 2.1 Target locus design used to isolate genome-wide data (see Chattopadhyay et al. [28] for further details). In brief, we first selected exons from the little brown bat (*Myotis lucifugus*) genome that are more than 200 bp long and are conserved across bats. Following this, we identified these exons in the in-house generated genome of the lesser short-nosed fruit bat (*Cynopterus brachyotis*). For every such exon in *C. brachyotis* genome, we also retrieved sequence data 500 bp upstream and downstream; each of these intron-exon-intron segments was considered one locus for the study

amplified using sequence capture protocols and sequenced on an Illumina HiSeq4000 platform (see Chattopadhyay et al. [28] for further details).

We generated over 634 million reads and retained approximately 483 million reads after clean-up (removal of adapters, low-quality reads, PCR duplicates) [28]. Historic samples carried characteristic signatures of DNA damage: excessive cytosine to thymine substitutions at the 5′ end and guanine to adenine substitutions at the 3′ end. All reads from these historic samples were rescaled and trimmed using map-Damage 2 [41]. The cleaned reads were processed in two ways: first, by generating sequence data for all 1184 target loci (using HybPiper pipeline 1.2 [42] and, second, by aligning these reads directly to the *C. brachyotis* genome and mining genome-wide single-nucleotide polymorphisms (SNPs) (ANGSD [43]). This dual approach helped us to generate diversity estimates informed by over a million base pairs of sequence data and thousands of SNP loci. Our analyses required genetic markers that are unlinked (i.e. have independent evolutionary histories), are selectively neutral (i.e. do not directly affect the fitness of the organism and are therefore not under selection) and have not undergone recombination. We checked our data for signatures of these evolutionary processes and pruned loci identified in this screening, retaining 874 loci for sequence-based analysis (990,087 bp) and 24,782 SNPs (see Chattopadhyay et al. [28] for additional details).

2.1   *Decline in Genetic Diversity*

We compared genetic diversity and inbreeding coefficients of the historic and contemporary populations of *C. brachyotis*. For the sequence data, we estimated the number of variable sites, number of parsimony informative sites, proportion of variable sites, and proportion of parsimony informative sites using AMAS [44]. We observed significant differences in genomic diversity estimated for all four summary statistics, with higher genomic diversity in historic populations than in contemporary ones (Fig 2.2a–d). We used the SNP data to measure internal relatedness, homozygosity by loci, proportion of heterozygous loci, and standardised heterozygosity relative to mean expected heterozygosity, using the R package GENHET [45, 46], pairwise relatedness using COANCESTRY 1.0.1.7 [47], and inbreeding coefficients with PLINK 1.9 [48]. We also generated estimates of genome-wide probability of heterozygosity per individual in ANGSD for all individuals. All SNP-based summary statistics other than homozygosity by loci were significantly different between historic and contemporary populations (Fig. 2.2e–k). All summary statistics suggested that the contemporary population is genetically impoverished relative to the historic sample, with higher inbreeding coefficients and higher pairwise relatedness between individuals (Fig. 2.2).
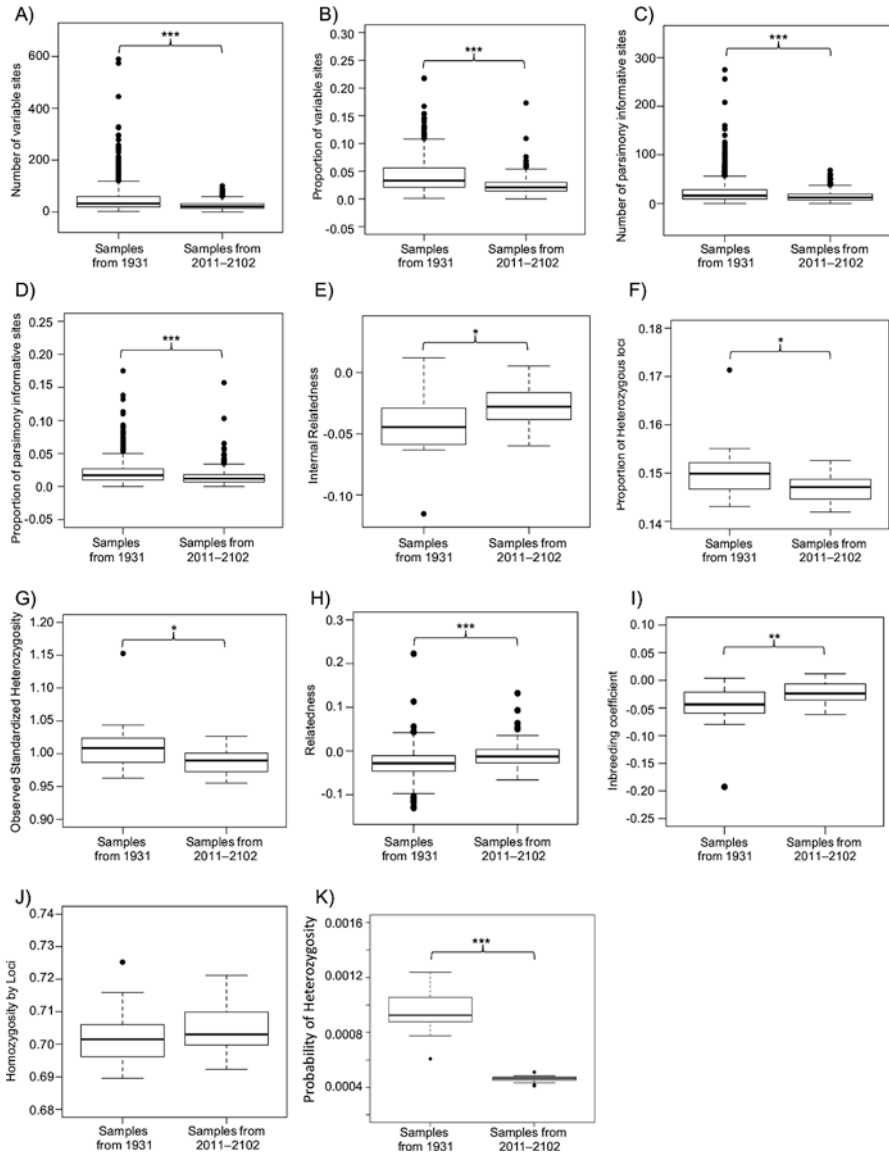
Fig. 2.2 Comparison of genetic diversity estimates for historic and contemporary samples of the lesser short-nosed fruit bat (*Cynopterus brachyotis*) from Singapore based on the sequence dataset (A–D) and SNP dataset (E–K). * denotes *p* values less than 0.05, ** denotes *p* values less than 0.01, and *** denotes *p* values less than 0.001

Our observations illustrate an overall reduction in genetic diversity over the past ~90 years within the Singapore population of *C. brachyotis* (Fig. 2.2). The Palaeotropical ecosystems of Asia have experienced large-scale destruction of forests and other natural habitats in the past century [7, 39, 49–51]. This region also harbours major biodiversity hotspots and cryptic diversity, with ongoing and regular discovery of species new to science [49, 50, 52]. However, the pace and extent of ongoing habitat destruction and the local effects of global climate change in Southeast Asia may drive many species to extinction even before they are described [7, 39, 49–51]. The island nation of Singapore is a microcosm of these challenges, with rapid urbanisation during the past century in conjunction with drastic deforestation driving local extinction of multiple species during the last five decades [7, 39, 53, 54]. *Cynopterus brachyotis* can be synanthropic but is also a keystone species in forests acting as both pollinator and seed disperser [55]. Declining genetic diversity in the Singapore population of *C. brachyotis* suggests population declines concurrent with urbanisation, implying that urbanisation can drive even ubiquitous species to become rare [28].

2.2   Drastic Population Bottleneck Coinciding with Urbanisation

The correlation between low genetic diversity and extinction is well established in the scientific literature; genetic factors partly predict the viability of small populations [56, 57]. Accumulation of deleterious mutations and overall loss of genetic diversity in some isolated populations have also been speculatively linked to local extinctions [31, 34]. Temporal sampling allows the empirical evaluation of genetic diversity loss over time [28–30, 58], and reduced genetic diversity between historic and contemporary samples implies fluctuations in population size. Comparisons of ancient and contemporary diversity have revealed demographic trends over long periods of time (e.g [58].). However, application of temporal sampling to understand the potential effects of the Anthropocene on the genetic diversity of wild populations is a relatively new approach [28, 30, 59, 60].

To understand the effects of human-mediated changes on the evolutionary trajectory of the Singapore population of *C. brachyotis*, we assessed support for competing models of demographic history. We constructed six different scenarios of population decline, including gradual decline, population bottlenecks (i.e. rapid, dramatic decreases in population size), and a combination of both decline and bottlenecks. We also included a model of constant population size and a model for gradual population expansion. We used the site frequency spectrum (SFS) for demographic reconstructions in fastsimcoal 2.6.02 [61] to compare these eight models (see Chattopadhyay et al. [28] for further details). We used a temporal sampling approach for demographic reconstructions as it can robustly identify bottlenecks under most circumstances compared to only analysing modern samples [29].
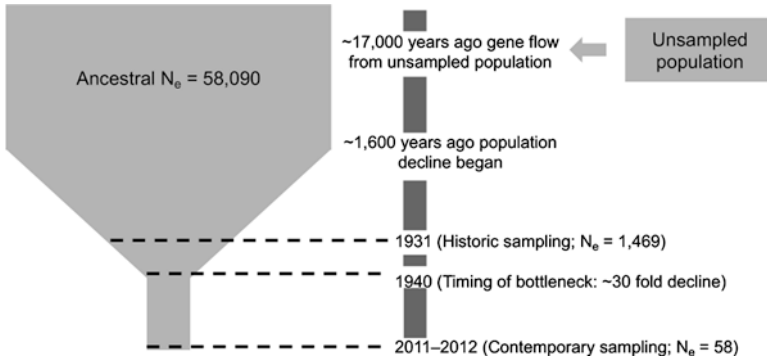
Fig. 2.3 Visual representation of the best fit model of demographic history of the Singapore population of the lesser short-nosed fruit bat (*Cynopterus brachyotis*)

We observed that the best model was the most complex one (Fig. 2.3), positing historic gene flow from an ancestral, unsampled population and a continuous decline that started 195 generations ago (range: 63 to 507 generations ago). Assuming a generation time of 8 years for *C. brachyotis*, this suggests that this decline started approximately 1600 years ago. This model also estimated a very recent bottleneck at nine generations ago (~1939, but range 2–11 generations ago).

2.3 Common Species Are Not Immune to the Effects of Fragmentation

Our demographic models provide strong evidence of direct impacts of urbanisation on natural populations, which may also apply to wildlife in other rapidly changing landscapes. The estimated timing of the recent bottleneck in the Singapore population of *C. brachyotis* coincides with the advent of the Anthropocene [1], a period of rapid urbanisation and drastic decline of forest cover in the island nation [7, 39, 54]. Ongoing efforts to promote habitat regeneration in Singapore may support the recovery of this and other affected populations, and long-term monitoring of these populations can assess the impact of such efforts.

The severity of the estimated bottleneck is considerable, as the nearly 30-fold decrease (range: 3–96-fold decline) is likely detrimental to population viability (Fig. 2.3). Bottlenecks increase a population's vulnerability to stochastic events increasing the probability of local extinctions [56, 57, 62]. Loss of genetic diversity and population bottlenecks due to human interference has been documented in many endangered vertebrates [5, 31, 56]. Our results build on this literature by demonstrating these effects in a common species in response to urbanisation.

The low genetic diversity and effective population size of *C. brachyotis* in Singapore (Fig. 2.3) raise concerns about the long-term viability of this island

population and provide a sobering example of the effect that urbanisation may have on wildlife. Human-mediated habitat alterations are not only severely detrimental to the survival of rare species but also affect common species coexisting closely with humans [28]. The Anthropocene, the epoch of human dominance, is synonymous with devastating defaunation due to human activities [1, 2, 4]. A recent study showed declining population sizes and shrinking ranges in ~9000 vertebrates, including common species, over the last century [6]. These ongoing declines imply an accelerated pace of genetic impoverishment that can interact with small population sizes to increase extinction risk [6]. Our study illustrates how modern sequencing technologies and genomic analyses can untangle the patterns and processes driving diversity loss, elucidating its causes and consequences in the medium and long term. Our study also illustrates the value of museum collections in enabling temporal sampling and comparisons of pre- and post-urbanisation populations, to quantify the true effects of the Anthropocene. We particularly acknowledge the value of museum collections that have been built by the massive efforts of various collectors in the past.

3   Conclusion

Taken together, our observations from bats provide insights into the effects of human-induced climate change and landscape modifications, particularly urbanisation, on resident wildlife. In a synthesis of multiple studies, we found that bats are particularly sensitive to climatic fluctuations and habitat loss. Our integrative research paradigm connecting past climate and habitat alterations with genome-scale data allowed us to identify detailed evolutionary patterns of natural populations from present diversity to historical fluctuations going back millions of years. Our results add to the growing body of literature showing the threat posed to wildlife by human encroachment. They also provide direct evidence of genetic impacts of urban development, indicating that even common, synanthropic species are threatened by urbanisation and climate change. Fruit bats such as *C. brachyotis* are keystone species that play a primary role in pollination, germination, and regeneration of native tree species [55, 63]. As such, the decline of these bats may adversely affect not only the function of the ecosystems they inhabit but also their long-term ability to support populations of humans and other wildlife.

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5 Data Availability

The data used for this study are available on Sequence Read Archive (project accession number is PRJNA666066) and GenBank (genome accession ID: GCA_009793145.1).

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