Philippine Journal of Science
150 (S1): 587-596, Special Issue on Biodiversity
ISSN 0031 - 7683
Date Received: 19 Nov 2020

Population Genetic Structure of *Eonycteris robusta* from Luzon Island

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Population genetics has been widely employed in conservation studies to assess genetic diversity, gene flow, and population structure. This information can be used to understand the population's fitness. The Philippine dawn bat *Eonycteris robusta* is a nectarivorous fruit bat that is ecologically important because of its capability to pollinate trees. It is categorized as "Vulnerable" in the Philippine Red List and the IUCN (International Union on the Conservation of Nature) Red List. Fragmentation and loss of forest habitats along with biogeographical factors might impact gene flow and affect the genetic diversity of this species. Thus, a population genetic study was done to assess gene flow and population structure of this species from two sites in the island of Luzon: Palanan, Isabela and Puning Cave, Doña Remedios Trinidad, Bulacan. Ten (10) microsatellite markers were developed from the whole genome of *E. robusta* and these were used for genotyping individuals. Observed heterozygosity (H_o) was generally lower than the expected (H_e), suggesting loss of heterozygosity. The inbreeding coefficient values (F) across all loci were greater than 0.5, which meant that mating between closely related individuals was frequent. A low level of gene flow was detected between Palanan and Puning Cave with the presence of structuring (F_{st} = 0.190 ± 0.003; N_m = 1.068 ± 0.020). Deviations from the Hardy-Weinberg equilibrium (HWE) were also observed for most of the loci tested. Clustering was also evident in the principal coordinates analysis (PCoA) plots, showing a distinct cluster of Puning Cave samples separate from Palanan, Isabela. This study has shown that within island clustering is possible, which may be driven by ecological factors such as discontinuity of available habitats for *E. robusta* or by biogeographical features like distance and topography.

Keywords: conservation genetics, fruit bats, microsatellite markers, population genetics

INTRODUCTION

Fruit bats act as excellent pollinators and seed dispersers in forest ecosystems because of their ability to cover large distances (Corlett 2017), making them key drivers of forest growth and regeneration. Most fruit trees in the forest heavily rely on fruit bats for propagation (Fujita and Tuttle 1991). Among these are early successional trees that are important species for forest regeneration such as *Ficus*, *Muntingia*, *Macaranga*, and *Melastoma* (Muscarella and Fleming 2007), and economically important trees that are sources of timber and fruits (Fujita and Tuttle 1991). Some tree species rely on fruit bats for the increased likelihood of seed germination; those ingested by fruit bats were more likely to germinate compared to those ingested by other animals (Hodgkinson 2003). Local extinctions of

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frugivorous bats, therefore, have negative impacts on forest regeneration (Hamann and Curio 1999).

Eonycteris robusta (the Philippine dawn bat) is a species of interest because it is endemic to the Philippines. It is a cave-dependent species and feeds only on nectar, making it entirely reliant on habitats that have both caves and forests (Heaney *et al*. 2016). The IUCN Red List and the recently updated Philippine Red List of Threatened Wild Fauna classified *E. robusta* as "Vulnerable" (BCSP 2020). It is projected that this species' populations are undergoing a decline, being rarely caught in field surveys (Heaney *et al*. 2016; Gonzalez *et al*. 2018). Declining populations and the possibility of decreasing genetic diversity may pose a problem for the survival of this species as both may result in inbreeding depression (Keller and Waller 2002).

Conservation studies often employ population genetics to evaluate the fitness of a population (Segelbacher *et al.* 2010). In recent decades, microsatellite markers such as simple sequence repeats or short tandem repeats (STR) have been preferred due to the advent of technologies that allowed for the exploitation of such genomic regions and because of their utility in uncovering demographic history (Campbell *et al*. 2006). These markers are inherited in a codominant fashion, hypervariable, and are very abundant in both coding and non-coding regions of the genome (Abdul-Muneer 2014).

The movement of an organism in an area can be inferred by detecting differences between populations through genotyping and clustering methods (Frankham 2018). Populations from areas where the movement of organisms can occur freely are likely to share genetic similarities because of high gene flow between them (Yamamichi and Innan 2012). Gene flow between populations is likewise affected by factors that limit the migration of individuals, be it natural (*e.g.* geographical barriers, seasonal variation, mobility of organisms, habitat availability, and food sources) or anthropogenic in nature (*e.g.* habitat fragmentation and other disturbances caused by human activities). Small, isolated populations may suffer the consequences brought about by limited migration and gene flow such as loss of genetic diversity, accumulation of harmful mutations, and reduced evolutionary potential (Frankham 2018). This makes population genetics studies an important facet of conservation efforts, as it can direct endeavors into specific areas or populations that could possibly need intervention.

Conducting population genetic studies on *E. robusta* can offer preliminary insights on the gene flow and population structure of an endemic forest- and cave-dependent species. This can contribute to the existing conservation measures in the Philippines by providing baseline information on how the gene flow of a species' populations reflects the current condition of lowland forests. Here, we aimed to assess the population genetic structuring and gene flow of *E. robusta* between two sites in Luzon Island (Figure 1) through the characterization of their microsatellite diversity. Island endemic species such as *E. robusta* are thought to experience inbreeding because of their inherently isolated and small population sizes (Frankham 1997; James 1970). Given most forests' fragmented condition in the Philippines and the topographical features that separate the two sites, population structuring should be present to a certain degree – similar to previous studies of another Philippine endemic fruit bat (Roberts 2006a; Peterson and Heaney 1993).

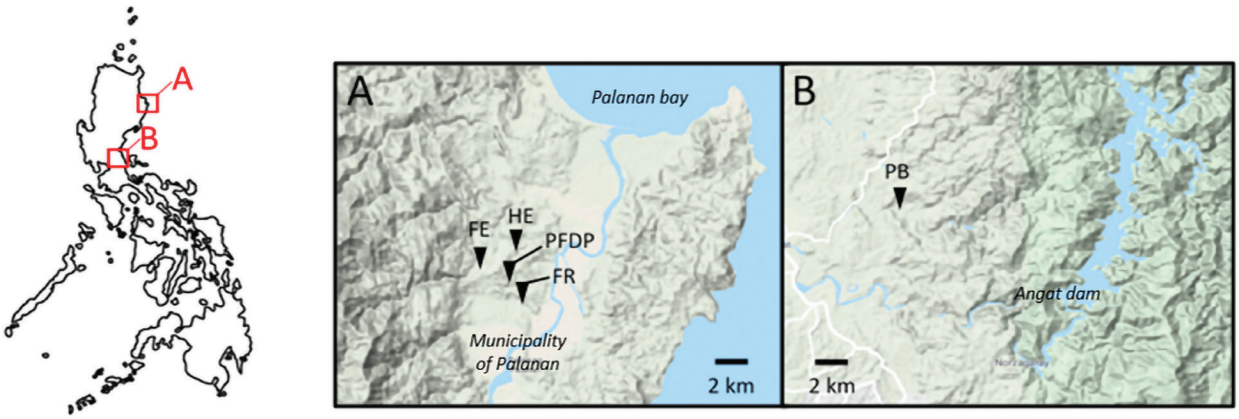


Figure 1. Location of sampling sites for this study: A) four in Palanan, Isabela [Forest Edge (FE), Palanan Permanent Forest Dynamics Plot (PFDP), Forest Ridge (FR), and High Elevation (HE) (Google Inc.)] and B) one in Puning Cave, Doña Remedios Trinidad, Bulacan (PB) (Google, Inc.). The map of the Philippine archipelago was downloaded from the Philippine Geographical Information System (PhilGIS).

MATERIALS AND METHODS

Study Site

The study covered five sampling locations from two sites in Luzon (Bulacan and Isabela) (Figure 1). These locations were selected to determine how gene flow of *E. robusta* behaved among closely situated lowland forest sites in Palanan (within 2 km away from each other) and whether gene flow would be found with the more distantly located site in Puning Cave (~ 275 km from Palanan). The four locations in Palanan, Isabela were named as forest edge (FE), Palanan permanent forest dynamics plot (PFDP), forest ridge (FR), and high elevation (HE). The characteristics of each site are summarized in Table 1.

Sample Acquisition

Wing patagium punches were obtained from the Biodiversity Research Laboratory (BRL), Institute of Biology, University of the Philippines (UP) Diliman. Field sampling was done by the BRL staff with permits issued by the Department of Environment and Natural Resources (Gratuitous Permit Nos. III-2015-01 and 2017-07) with bats captured and handled in accordance with animal care and use guidelines established by the American Society of Mammalogists (Sikes and Gannon 2011). Captured individuals were identified by Lystra Zyrill A. Dayapera and Christopher John A. Pueblo of the BRL in the field using the keys by Ingle and Heaney (1992). A total of 117 individuals from the Palanan sites (FE = 30; PFDP = 30; FR = 27; HE = 30) and 17 individuals from Puning Cave were used in this study. Morphometric data such as body mass and lengths of the forearm and hindfoot were also measured during bat capture as supplemental data for species identification (data not shown).

Whole Genome Sequencing, Microsatellite Mining, and Primer Design

DNA was extracted following the protocol of the PureLink® Genomic DNA Purification Kit from the muscle tissue of an individual *E. robusta* (tag: MRMD 2124) that was previously collected by the personnel of the BRL, UP Diliman, and identified by Dr. Mariano M. Duya. The concentration was noted using fluorometric methods to ensure that there was at least 40 ng/µl of DNA. The DNA Sequencing Core Facility–Philippine Genome Center (DSCF-PGC) was tasked with performing the whole genome sequencing. A DNA library was prepared by the SGCL-PGC using the TruSeq DNA PCR-free library preparation kit (Illumina, Inc.). The prepared DNA library sample was sequenced with the NextSeq 500 Platform (Illumina, Inc.) with paired read lengths of 150 bp for a total of 151 cycles with an output of 59.76 gbp. Sequencing quality was assessed through the Phred quality scoring and the average Q30 score of the completed NGS run for individual MRMD 2124 was 88.01%, which indicated good base calling and that the NGS data could be used for downstream analysis (*i.e.* STR prediction) (Richterich 1998; Ewing and Green 1998).

Whole-genome data assembly and primer design were done through the services of the Core Facility of Bioinformatics–Philippine Genome Center (CFB-PGC) using the Short Oligonucleotide Analysis Package *de novo* version 2 (SOAPdenovo2) (Luo *et al*. 2012). A K-mer sweep was done to choose the most effective K-value for assembling the genome. After assembly, the Quality Assessment Tool for Genome Assemblies (Gurevich *et al*. 2013) was used for quality checking. The assembled genome was also compared to available pteropodid whole

Table 1. Site descriptions of sampling sites covered by this study.

Location	Site name	GPS coordinates	Elevation (masl)	Remarks
Palanan, Isabela	Forest edge (FE)	17° 2' 44.196" N, 122° 22' 45.516" E	120	Near small patches of slash-and-burn subsistence farms and to the Dipogen River, where a nearby cave is situated (17° 4' 9.84" N, 122° 22' 13.44" E)
	Palanan forest dynamics plot (PFDP)	17° 2' 24.756" N, 122° 23' 8.088" E	99	Dominated by dipterocarp trees (Dipterocarpaceae) along with other major families (Meliaceae, Arecaceae, Puntrajivaceae, and Lauraceae)
	Forest ridge (FR)	17° 2' 8.952" N, 122° 23' 22.452" E	91	Located on a slope within a secondary lowland forest formation
	High elevation (HE)	17° 2' 50.208" N, 122° 23' 13.884" E	160	Relatively higher in elevation than other sites.
Doña Remedios Trinidad, Bulacan	Puning cave (PB)	14° 57 '44.4"N, 121° 5' 31.5"E	110	Located near Puning Cave and to an active open-pit mining site

genomes (*Pteropus vampyrus* and *Pteropus Alecto*). The genome of the congeneric *E. spelaea* (Wen *et al*. 2018) was not yet available when this study was being conducted.

STRs were predicted through the Microsatellite Identification Tool (Thiel 2003) and the sciRoKo (STR Classification and Investigation by Robert Kofler) version 3.4 (Kofler *et al*. 2007), which utilized the dictionary and slide approaches for predicting repeats, respectively. STRs predicted by both tools that were at least eight base pairs long were included in the STR list. The list of STR loci was filtered using the following criteria: a) only perfect STRs were included, b) mononucleotide repeats were removed, and c) the loci were at least 20 base pairs away from either end of the contig. Only STR loci that passed these three criteria were subjected to primer design.

Primers were designed using primer3 (Untergasser *et al*. 2012). Primers that had a sequence length of 17–25 bp (optimal length of 20 bp) were selected. The resulting amplicon had to be between 100–500 bp. The melting temp (T_m) was set between 53–60 °C with an optimal T_m of 55 °C. GC content was set to 20–80% with an optimal GC content around 50%. No homopolymer stretch longer than three base pairs were included in the primer sequences.

The primer pool was then filtered from an initial 49,363,864 primer pairs down to a more manageable size. The first step was to filter primers by repeat motif; only the di- and tri-nucleotide repeats were considered. The second step was filtration by amplicon size; those that would amplify less than 200 bp were removed from the list since smaller fragment sizes are hard to separate further in polyacrylamide gel electrophoresis. A list of 85 pairs was randomly selected from the filtered pool for synthesis. Primers were tested until a final batch of 10 pairs were selected for use in the study as population genetics studies on bats generally find this number to be enough (Hua *et al*. 2006; Shao *et al*. 2008; Storz 2000). The information on these primers is summarized in Table 2 along with GenBank accession numbers for the microsatellite sequence amplified by each primer pair.

Genotyping and Population Genetic Analysis

Primers designed from the mined microsatellite markers were used for genotyping the *E. robusta* individuals collected from the different field sites (Figure 1). The same kit and extraction protocols were used to extract genomic DNA from their wing patagium samples.

Marker DNA fragments from the samples were amplified using the validated set of reverse and forward primers with the following PCR mix components: 7.305 µl nuclease-free water, 2.5 µl 5X MyTaq (Bioline) PCR buffer, 0.625 µl 5% dimethyl sulfoxide, 0.25 µl of 50 mM $MgCl_2$, 0.375 µl each of the 10 mM left and right primers, 0.07 µl of 5 units/µl MyTaq (Bioline) Taq DNA polymerase, and 1.0 µl of the extracted DNA (1.0–15.0 ng/µl). The PCR conditions had an initial T_m of 94 °C, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, ending with a final extension temperature of 72 °C for 5 min.

The resulting profiles were scored using GelAnalyzer2010A (Lazar and Lazar 2010) and raw data analyzed using GenAlex 6.502 (Peakall and Smouse 2006) to determine population genetics summary statistics such as N_a (number of alleles), N_e (number of effective alleles), H_o, H_e, deviations from HWE, N_m (gene flow value), molecular variance, F, and F_{st} (fixation index). The same software

Table 2. The following microsatellite loci were amplified using primers summarized in this table. Product sizes are estimates from the primer design step.

Species: *E. robusta*						
Locus name	Locus number	Repeat motif	Left primer	Right primer	Estimated product size (bp)	GenBank accession number
			Seq (5'-3')	**Seq (5'-3')**		
Erb1	1	$(ATC)_{11}$	TGCTTGGGAAGGTCACTTGC	GCAGCTGACACAGGATAGGT	273	MW619730
Erb2	2	$(TTG)_{12}$	AGGCTCCAGAGACTTCCAAA	CCCAAGCAACTTCACCATACA	222	MW619731
Erb3	3	$(ATT)_{12}$	GCTGTGTGAACATGGGCAAG	AAGGGCAGGTGCTTCTGTAA	268	MW619732
Erb4	4	$(TC)_{15}$	GGACCACAGGTAATCACGGG	CTCGGTCATAAATCCCTGGCA	332	MW619733
Erb5	5	$(ATT)_{11}$	TCCTCACCAACACTTGCTGT	AGACTCCCAAGGGTAGCACT	290	MW619734
Erb6	6	$(GT)_{14}$	GGCTGTGGTGCATGGAGATA	TGGCATAGGTGGCATCTGTT	288	MW619735
Erb7	7	$(AT)_{11}$	TCCGGAAACCTCTCAGGTCA	TTCAGAGGCTGACGTTTGGG	263	MW619736
Erb8	8	$(AC)_{10}$	AGCCGATATCATCAGGAGAGGA	GGGTTGCAATTCTCTGCTTTCA	454	MW619737
Erb9	9	$(AC)_{20}$	ACCATGAATTCCACAAGGTGA	ACCTTGGCAGCTTCCTGAAA	444	MW619738
Erb10	10	$(AC)_{11}$	TTTCTTCGCACCTTCCAAAT	TGTTCTGCGTACTTAATGGGT	282	MW619739

was also used to perform PCoA. The polymorphic information content (PIC) and proportion of null alleles (F_{null}) were also determined using the CERVUS software ver.3.0.7 (Slate *et al*. 2000). Data from Palanan sites were then pooled and compared with data from Puning Cave.

RESULTS

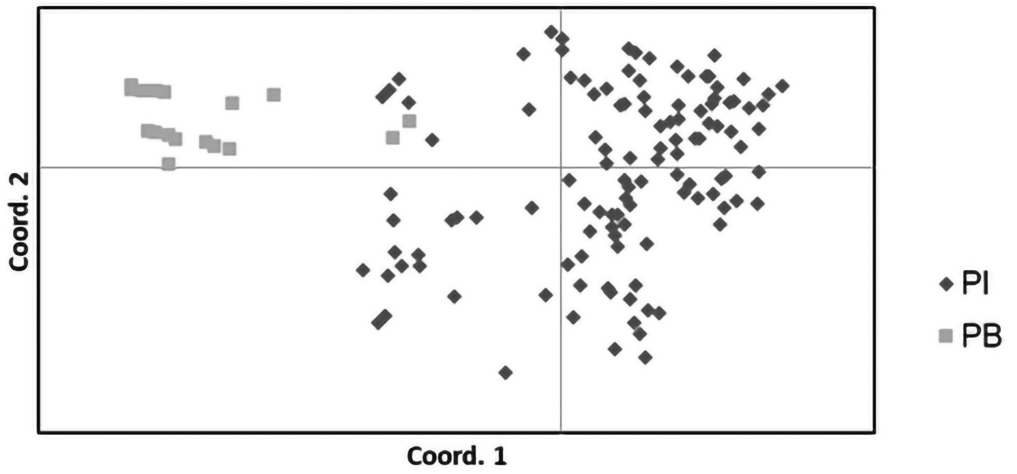
The 10 microsatellite loci used for this species exhibited varying N_a ranging from 2–19, with Locus 4 having the highest N_a and N_e in both Palanan sites and Puning Cave (Table 3). The PIC of most of the loci was high, with Loci 1–4 being consistently above the threshold (PIC > 0.5) (Table 3), making it useful in doing linkage-maps (Botstein *et al*. 1980; Nagy *et al.* 2012). Most of the values for H_o were lower than the expected heterozygosity (H_e) (Table 3), suggesting loss of heterozygosity. The average F values were 0.626 ± 0.087 in Palanan and 0.605 ± 0.128 in Puning Cave (Table 3). Deviations from HWE were detected in almost all loci (Table 3). There was also a high frequency of null alleles in four loci ($F_{null} > 0.5$) (Table 3).

Little population sub-structuring and high gene flow were observed within Palanan, Isabela with an average F_{st} = 0.091 ± 0.011. At the same time, there was high population structuring and low gene flow between Palanan and Puning Cave with an average F_{st} = 0.190 ± 0.003 ($p <$ 0.01). The smaller number of migrating individuals (N_m = 1.068 ± 0.020) between Palanan and Puning Cave supports this finding that there is a low level of gene flow across the two, compared to the higher values among the sites within Palanan (N_m = 2.756 ± 0.414) ($p < 0.01$).

Variance within populations (24%) was found to be higher than the variance between populations (20%). The population structuring is demonstrated in the formation of a noticeable cluster of Puning Cave individuals away from the Palanan cluster, as shown in the PCoA plot (Figure 2).

Table 3. Population genetics summary statistics for each sampling site: sample size (N), number of alleles (N_a), number of effective alleles (N_e), polymorphic information content (PIC), observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F), and global probability for null alleles [$F_{(null)}$]. Also shown are deviations of each locus from HWE using chi-squared test: *$p <$ 0.05 and ***$p < 0.001$.

Site	Locus number	N	N_a	N_e	PIC	H_o	H_e	HWE	F	$F_{(null)}$ global
Palanan, Isabela	1	112	15	7.7	0.856	0.018	0.841	***	0.979	0.949
	2	115	15	5.5	0.795	0.296	0.752	***	0.639	0.486
	3	116	17	8.6	0.872	0.336	0.873	***	0.619	0.500
	4	112	33	17.2	0.939	0.170	0.931	***	0.820	0.704
	5	114	14	9.9	0.890	0.140	0.849	***	0.844	0.736
	6	113	6	2.9	0.583	0.442	0.542	***	0.320	0.274
	7	116	15	4.5	0.752	0.155	0.592	***	0.800	0.664
	8	115	4	1.5	0.305	0.339	0.441	***	0.042	0.009
	9	112	9	2.8	0.599	0.286	0.649	***	0.560	0.460
	10	116	11	4.5	0.742	0.284	0.810	***	0.633	0.483
	Mean	114	14	6.5	0.733	0.247	0.728	–	0.626 ± 0.087	–
Puning Cave, Bulacan	1	17	6	4.4	0.741	0.059	0.773	***	0.924	
	2	17	8	6.4	0.824	0.294	0.843	***	0.651	
	3	17	8	7.1	0.844	0.059	0.860	***	0.932	
	4	15	8	5.6	0.801	0.133	0.822	***	0.838	
	5	17	2	1.4	0.248	0.118	0.291	*	0.595	
	6	16	4	1.6	0.331	0.125	0.363	*	0.656	
	7	16	3	1.3	0.215	0.250	0.227	ns	–0.103	
	8	17	2	1.3	0.186	0.235	0.208	ns	–0.133	
	9	17	2	1.3	0.186	0.000	0.208	***	1.000	
	10	17	5	2.4	0.521	0.176	0.576	***	0.694	
	Mean	17	5	3.3	0.490	0.145	0.517	–	0.605 ± 0.128	



Axis	1	2	3
%	9.23	7.98	5.45
Cumulative %	9.23	17.21	22.65

Figure 2. PCoA plot showing the clustering of the *E. robusta* individuals using their genetic distances. Percent variations explained by the first three axes are shown below the plot. Sampling sites are indicated by the shape and shade of plot points. PI stands for Palanan while PB stands for Puning Cave.

DISCUSSION

Both the loss of heterozygosity and high values of *F* are typically observed in bottlenecked populations (*e.g.* insular or isolated populations) (Frankham 1997; James 1970). With *E. robusta* being an island endemic and threatened species, it might be possible that the observed *F* and loss of heterozygosity in the surveyed populations were because of a recent founder effect bottleneck. Loss of heterozygosity may be correlated with inbreeding although this correlation may be weak (Slate *et al.* 2004; Pemberton 2004; Grueber 2008). The observed deviations from HWE may also suggest inbreeding, but the power of HWE for detecting inbreeding is small and must be carefully considered especially when null alleles are present (Pemberton *et al*. 1995). Furthermore, we observed varying values of *F* across the loci, and this could mean that the observed loss in heterozygosity was due to population admixture rather than inbreeding (Robertson and Hill 1984).

Having an F_{st} closer to 0 means no structuring and high gene flow, while $F_{st} = 1$ means complete isolation and no gene flow between two populations (Yamamichi and Innan 2012). Having an $N_m > 0.5$ meant that allele fixation due to genetic drift is not likely to happen (Wolf and Soltis 1992). The lower level of variance between populations could mean that there is a possibility of gene flow between the Palanan sites and the Puning Cave site (Mills and Allendorf 1996). On the contrary, highly isolated populations would exhibit higher variance between populations than within populations (Rosenberg *et al*. 2002). This presence of gene flow between the sites further refutes the possibility that inbreeding is present in these populations.

The high gene flow within Palanan was expected because of the proximity of the sampling sites to each other (within 2 km). Although the exact cave dwellings of the sampled individuals were not known, it is very likely that there is connectivity between the cave colonies in the vicinity of the sampling areas facilitated by the flight capabilities of *E. robusta*. Other cave-dwelling fruit bats such as *Rousettus amplexicaudatus* and *E. spelaea* are known to travel long distances in search of food: *E. spealea*'s commuting distance ranges from 17.9–38 km (Acharya *et al*. 2015; Start 1975) while *R. amplexicaudatus* can reach up to 25 km (Heaney *et al*. 2016). It should be possible for *E. robusta* to cover the same distance as they share a very similar body size with *E. spelaea* but with a higher wing aspect ratio and wing loading, which are directly correlated with the distance that they can travel (Duya *et al.* 2017).

The availability of habitats and food sources also play a role in the population structuring of this species. Bats of the genus *Eonycteris* exclusively feed on nectar and roost in caves (Gonzalez *et al*. 2018; Hodgkinson 2003). Current records of this species indicate that they are absent from surveys in agricultural and urban areas but are found

in primary and secondary forests in the vicinity of their roosting cave (Heaney *et al.* 2016). This may mean that their preference for caves and forested habitats could restrict the movement of *E. robusta* (Duya *et al*. 2020). This could hinder gene flow between separate populations to some extent. In the case of the sampling sites, those from Palanan are surrounded by agricultural activities (*e.g.* farmlands, slash-and-burn patches) while Puning Cave in Bulacan is near an active mining site and built-up areas.

Roost fidelity could also contribute to the gene flow of this species by limiting their movement among caves within the proximity of their food source, although no studies on roost fidelity have been done yet on *E. robusta*. Roosting behavior studies on the related *E. spelaea* show strong roosting fidelity and the same was observed in other nectarivorous bats (Acharya *et al*. 2015). This was also the case for *Rousettus aegyptiacus*, another cave-dwelling fruit bat, as a high degree of relatedness between bats was observed among nearby roosting caves (Bachorec *et al*. 2020).

The low gene flow observed between the Palanan and Puning Cave sites could be attributed to the distance between them (~ 275 km), which could be possibly greater than the commuting travel of *E. robusta*. Another important geographic feature is the Sierra Madre mountain range that borders the eastern side of Luzon, facing the Pacific Ocean. The Palanan sites were situated on the eastern side of the Northern Sierra Madres, while Puning Cave is situated on the western side of the southern Sierra Madre Range. This topographical separation, along with the distance, could explain the observed limited gene flow and differentiated population structuring. These two segments of the Sierra Madre are separated by the Sierra Madre biogeographic boundary (where the Mingan Mountains are situated) and are considered as distinct biotic regions of Luzon (Vallejo 2014). However, the magnitude of this separation is likely less pronounced in volant taxa compared to non-volant taxa, which is why the two populations we studied were not completely isolated.

Previous population genetic studies in other pteropodids (*Cynopterus brachyotis*, *Macroglossus minimus*, *Ptenochirus jagori*, and *Haplonycteris fischeri*) shared similar results to our study. These species provide interesting points of comparison as the first two are widespread and the latter two are endemic to the Philippines. In the endemic taxa with a strong preference for forested areas (*H. fischeri*) (Heaney *et al*. 2016), higher population structuring and more limited gene flow relative to the results of the two widespread taxa were reported (Peterson and Heaney 1993; Roberts 2006a). However, in the other Philippine endemic (*P. jagori*), less population structuring was reported relative to the widespread taxa (Roberts 2006b). The species' tolerance for disturbance in both forested and developed areas may allow it to utilize man-made structures in lieu of natural caves (Heaney *et al*. 2016), thus allowing it to cross otherwise unsuitable habitat to maintain contiguous gene flow across the landscape.

CONCLUSIONS

This is one of the few studies examining the population genetics of fruit bats in the Philippines. Here we developed and utilized microsatellite markers to evaluate the gene flow and population sub-structuring of the *E. robusta* populations from Palanan and Puning Cave. It was found out that there was little gene flow and high sub-structuring between Palanan and Puning Cave, suggesting that within island population structuring was observable for this species. This population structuring might have been brought about by the discontinuity of habitats or the distance and topography between the two sites.

The whole-genome sequence generated in this study can also be used for other purposes, such as mining other features through bioinformatic means (*e.g.* mitochondrial genome extraction and assembly). However, the read-length provided by the Next-seq 500 platform was not long enough to address the issue of the repetitive nature of the fruit bat genome; hence, the sequences may not be useful for doing whole-genome analysis and it is recommended that longer read lengths be used in future whole-genome studies on other fruit bats.

While the microsatellite markers are very convenient tools in doing conservation studies, especially in those concerned with threatened taxa, there are certain disadvantages that could limit their use in doing conservation genetics. The mechanism on how length polymorphisms arise is hard to model compared to that of single nucleotide polymorphisms (SNPs), which can be easily analyzed by widely used nucleotide substitution models (Putman and Carbone 2014). Their high mutation rate also makes their length-based characterization prone to errors caused by homoplasy and may cause underestimation of allele diversity (Barthe *et al.* 2012). This makes microsatellites not an ideal choice in demonstrating how mutation gives rise to genetic diversity in populations. Additionally, F_{st} and its related values are often depressed (*i.e.* observed to be lower than what is to be expected) at high mutation rates (Putman and Carbone 2014) and this can be problematic when it comes to describing population structures using microsatellite markers. These disadvantages can be covered by analyzing microsatellites in conjunction with SNP data, which may yield more reliable inferences when it comes to areas where microsatellites lack power.

RECOMMENDATIONS

More available data on multiple fruit bat species from other islands of the Philippines will help determine what ecological and biogeographic features shape the patterns in the population structure we see in the landscape. These findings would be useful in guiding conservation efforts geared towards restoration of habitats and establishment of corridors that allow mobility between forest fragments, intact forests, and cave habitats not only for *E. robusta* but also for other taxa that share similar habitat requirements. For instance, when establishing corridors meant to connect isolated habitats with larger intact forests, it would be best to use gene flow data to evaluate if the establishment of the corridor were able to re-establish or even enhance the gene flow between the sites several years after. Gene flow data could also uncover already existing natural corridors that can be maintained and can serve as models in making artificial ones; we can determine and mimic the species assemblage of the flora that facilitates the optimal movement of organisms. Population genetic data can also help in determining highly diverse source populations, from which gene flow can be established with genetically depauperate ones, thus allowing some form of genetic rescue. These are just some of the benefits that can be gained should we confront conservation problems using the "genes to ecosystems" approach.

ACKNOWLEDGMENTS

We would like to thank the funding agencies responsible for funding the project covering this study: The Department of Science and Technology–Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development; and the Emerging Science and Technology Program and the Emerging Interdisciplinary Research of the Office of the Vice President for Academic Affairs (EIDR-C06-022.4). We thank the Biodiversity Research Lab of the Institute of Biology, UP Diliman for identifying and providing the samples used in this study. We also thank the DSCF-PGC and the CFB-PGC for the services they provided in this project. We also thank to Dr. Jonas Quilang, Dr. Carmela Española, and Dr. Ma. Josefa Pante for being part of the review process of this paper as part of a master's thesis. We are also grateful to all the high school and college interns/trainees in their respective internship programs who worked with us in performing some of the experiments, especially to Mr. John Vincent Navalan and Ms. Louise Angeli Asnan.

STATEMENT ON CONFLICT OF INTEREST

There are no conflicts of interest relating to the funding entities and the results of this study.

NOTES ON APPENDICES

The complete appendices section of the study is accessible at http://philjournsci.dost.gov.ph

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