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GENETIC DIVERSITY OF THE CAVE ROOSTING DUSKY FRUIT BAT, *Penthetor lucasi* FROM SARAWAK

YUVARAJAN MANIVANNAN[1*], MOHD RIDWAN ABD RAHMAN[2], ROBERTA CHAYA TAWIE TINGGA[2] and FAISAL ALI ANWARALI KHAN[1*]

[1]*Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak*
[2]*Centre for Pre-University Studies, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak*
[*]*E-mail: yuvanyuv7@gmail.com; akfali@unimas.my*

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ABSTRACT

Previous studies have revealed a high genetic divergence in *Penthetor lucasi* population in Sarawak, indicating it may consist of multiple genetic lineages. However, these studies are not accompanied by morphological data from the studied population suggesting this may be an effect of isolation by distance, especially with small sampling coverage. Here, we provide information based on D-loop sequence analysis from 32 individuals of *Penthetor lucasi* collected from four different regions in Sarawak. Our results revealed a high haplotype diversity and low nucleotide diversity, suggesting that these populations are possibly recovering from a recent geological and climatic event. However, neutrality test and mismatch distributions showed long-term population stability with no population subdivision observed among the studied populations. The moderate to high level of gene flow found in this study indicates that *Penthetor lucasi* population is likely panmictic. Meanwhile, the low level of genetic divergence value among and within populations could account for the absence of species complexity in this study. Our result highlighted the importance of sampling coverage in proper assessment of species diversity especially in species with wide distribution.

Key words: Complexity, D-Loop, divergence, gene flow, panmictic

INTRODUCTION

Bats from the Order Chiroptera represent the second most diverse order from the class Mammalia. In term of global diversity, a total of 131 species of bats from different feeding diet is known to be from Malaysia (Francis 2008; Payne *et al.,* 1985; Phillips & Phillips, 2016). From this, 81 species of insectivorous bats and 18 species of frugivorous and nectarivorous bats can be found in Sarawak (Phillips & Phillips, 2016). Besides controlling global insect population, bats play an important role in balancing the ecosystem through pollination and seed dispersal in the tropical region (Fujita and Tuttle 1991). However, anthropogenic disturbances such as cave closures and forest clearings have affected much of the bats' roosting and foraging activities (Mildenstein *et al.,* 2005; Struebig *et al.,* 2007; Epstein *et al.,* 2009). This leads to bat population clearing or fragmentation which may result in bat population decline or segregation that can potentially intrude the overall forest ecosystem (Russel, 2005). Therefore, studies involving bat population structure and genetic diversity are important to indirectly inform the sustainable management of caves and forest (Mohd-Ridwan & Abdullah, 2012).

Population genetic studies provide means to address the above issues through the understanding of the genetic diversity of a population affected by environmental changes and habitat loss (Clark *et al.,* 2008; Pruett *et al.,* 2008; Renshaw *et al.,* 2006; Steiper, 2006; Bryja *et al.,* 2009). These studies also inform the genetic make-up of a population over time by revealing which gene is constant and which gene has changed due to environmental factors or other mutation factors through generations (Burland & Worthington-Wilmer, 2001). This is crucial because these unique and rare genes within a gene pool can be an important source of natural selection

* To whom correspondence should be addressed.

to prevent extinction in a changing environment (Russel, 2005). Population genetic studies through nucleotide and haplotype diversity have revealed many important results that can be useful for conservation purposes in resolving species complexity and understanding gene flow between populations of bats (Aboim *et al.,* 2005; Russel *et al.,* 2005; Mohd-Ridwan & Abdullah, 2012; Larsen *et al.,* 2014). This information can also be used in distinguishing species within a complex (e.g. genetic structure in three species of whiskered bats (genus *Myotis*) during swarming; Bogdanowicz *et al*., 2012); interpreting past and present genetic knowledge (e.g. genetic variation within currently recognised *C. brachyotis*; Abdullah, 2003); demographic histories and possible migration patterns of bats (e.g. *E. spelaea and C. nusatenggara* from Indonesia Archipelagos) (Hisheh & Schmitt, 1998). Although bats have the ability to fly and cover more distance compared to non-volant small mammals, above studies showed that forest fragmentation can result in either continued or reduced gene flow in these bats' population. This further highlight the importance of population genetic studies in better understanding the ecology and behavior of an organism.

Dusky fruit bat, *Penthetor lucasi* is an exemplary species that is able to commute long distance for foraging like other fruit bats (e.g. *C. brachyotis* and *E. spelaea* which can travel up to 50 km per night for foraging) (Fukuda *et al*., 2009) and therefore serves as an important seed disperser in tropical rainforests (Feldhamer *et al.,* 1999; Mohd-Ridwan & Abdullah, 2012). *Penthetor lucasi* is a common fruit bat roosting in large colonies at most caves in Southeast Asia and is listed as Least Concerned by IUCN Red List of Threatened Species (Bates *et al.,* 2008). The previous study on *P. lucasi* using cytochrome b gene marker showed that this species has a high genetic divergence (Kimura 2-parameter ~ 5.0) within its populations (Anwarali *et al.,* 2008; Mohd-Ridwan & Abdullah, 2012). These findings suggest the possibility that *P. lucasi* may be comprised of multiple genetic lineages. However, these studies do not provide insights or were not able to provide enough information on the genetic variation and demographic history (Aboim *et al.,* 2005; Whittaker *et al.,* 2007; Gravel *et al.,* 2011; Saeidi *et al.,* 2013).

Therefore, our study aims to investigate the genetic variation and population structure of *Penthetor lucasi* in Sarawak using D-loop mitochondrial marker. This study explores genetic variation captured in Mohd-Ridwan & Abdullah (2012) by using material from almost all the sites studied by them (Kuching, Miri, and Sri Aman) with additional samples from central Borneo (Ulu Baleh) to close their sampling gaps within Sarawak.

MATERIALS AND METHODS

Area of sampling and field techniques

Samples were collected from four major populations representing Kuching (Gunung Gading and Wind Cave), Sri Aman (Engkilili), Kapit (Ulu Baleh) and Miri (Mulu and Niah) (Figure 1). The population grouping was made based on the distance between the localities (e.g. Localities with distance less than 150 km are considered as one population group). This is because, bats are known to travel around 50 km to 150 km in a single night for foraging (Fukuda *et al*., 2009 & Shilton *et al.,* 1999). A total of ten mist-nets were set below forest canopy across paths presumably bat's flyways. Captured bats were removed from the nets and kept into clean cloth bags until processed. All specimens were measured following standard museum protocols using digital calipers and weighed using Pesola balance springs. All specimens were identified based on Payne *et al.* (1985). Only selected specimens were processed as museum voucher specimen, where tissue samples were preserved immediately in 95% ethanol. Other samples were taken from the Zoological Museum of Universiti Malaysia Sarawak. A total of 27 new deposits and five museum voucher samples from the Zoological Museum of Universiti Malaysia Sarawak were used in this study (Table 1).

DNA extraction, polymerase chain reaction and sequencing

DNA extraction was performed using Cetyl Trimethyl Ammonium Bromides (CTAB) method (Grewe *et al.,* 1993). PCR was carried out using forward (GCTGAGGTTCTACTTAAACT; RodmtU) and reverse (GAGATGTCTTATTTAAGGGG; RodmtL) D-loop primers (Brown *et al.,* 2011). PCR was performed using the following thermal profiles: pre-denaturation at 95°C for 10 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 52.6°C for 1 minute and extension at 72°C for 1 minute with a final extension at 72°C for 10 minutes. Successful PCR products with high intensity were sent for DNA sequencing at a private laboratory (First BASE Company)

Genetic analysis

SEQUENCHER version 4.1.4 was used to display fluorescence-based DNA sequences and chromatograms of nucleotides bases. The multiple alignments of DNA sequence were done using CLUSTAL X version 1.81 (Thompson *et al.,* 1997). Further, the DNA sequences were checked and aligned by naked eyes. Molecular Evolutionary Genetic Analysis (MEGA) version 6.0 (Tamura *et al.,* 2013) was used to compute the pair-wise distance between population using Kimura-2-parameter

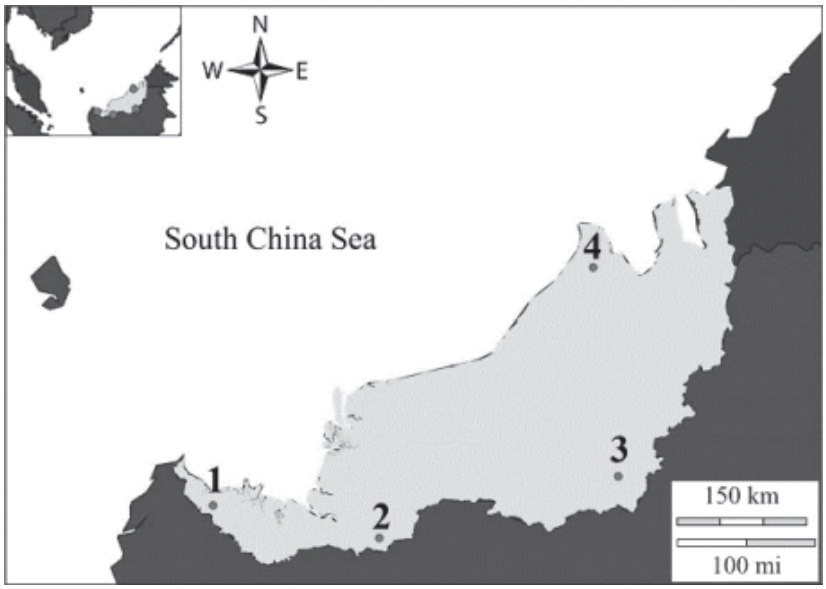

Fig. 1. Maps showing the populations of *P. lucasi* specimens needed for the molecular analyses. (1) Kuching; (2) Sri Aman; (3) Kapit; (4) Miri. Map was modified from QGIS software.

Table 1. List of samples used in this study

List	Samples	Field Number	Locality	Genbank Number
1	New deposit	GGNP15-115	Gunung Gading National Park	MH104848
2	New deposit	GGNP15-122	Gunung Gading National Park	MH104847
3	Museum voucher	MZU/M/2260	Gunung Gading National Park	MH104841
4	New deposit	UB15-37	Ulu Baleh Reserve Forest	MH104851
5	New deposit	UB15-40	Ulu Baleh Reserve Forest	MH104840
6	New deposit	UB15-41	Ulu Baleh Reserve Forest	MH104849
7	New deposit	UB15-42	Ulu Baleh Reserve Forest	MH104839
8	New deposit	UB15-43	Ulu Baleh Reserve Forest	MH104852
9	New deposit	UB15-44	Ulu Baleh Reserve Forest	MH104838
10	New deposit	UB15-45	Ulu Baleh Reserve Forest	MH104837
11	New deposit	UB15-48	Ulu Baleh Reserve Forest	MH104836
12	New deposit	MNP14-012	Mulu National Park	MH104834
13	New deposit	MNP14-020	Mulu National Park	MH104835
14	New deposit	MNP14-052	Mulu National Park	MH104833
15	New deposit	MNP14-067	Mulu National Park	MH104831
16	New deposit	MNP14-069	Mulu National Park	MH104832
17	New deposit	NNP14-023	Niah National Park	MH104830
18	Museum voucher	MZU/M/2250	Mulu National Park	MH104850
19	Museum voucher	MZU/M/2252	Mulu National Park	MH104829
20	Museum voucher	MZU/M/1957	Wind Cave Nature Reserve	MH104843
21	Museum voucher	MZU/M/1968	Wind Cave Nature Reserve	MH104842
22	New deposit	WCNR15-003	Wind Cave Nature Reserve	MH104846
23	New deposit	WCNR15-004	Wind Cave Nature Reserve	MH104845
24	New deposit	WCNR15-005	Wind Cave Nature Reserve	MH104844
25	New deposit	ENG15-68	Engkilili Reserve Forest	MH104828
26	New deposit	ENG15-71	Engkilili Reserve Forest	MH104827
27	New deposit	ENG15-72	Engkilili Reserve Forest	MH104826
28	New deposit	ENG15-73	Engkilili Reserve Forest	MH104825
29	New deposit	ENG15-75	Engkilili Reserve Forest	MH104824
30	New deposit	ENG15-76	Engkilili Reserve Forest	MH104823
31	New deposit	ENG15-118	Engkilili Reserve Forest	MH104822
32	New deposit	ENG15-119	Engkilili Reserve Forest	MH104821

(K2P) model (Kimura, 1980) to allow comparison with other similar studies. Haplotype tree inferred from D-loop gene was reconstructed using Maximum Likelihood (ML) method implemented in a phylogenetic analysis using MEGA v6.0 and the best-fit-model determined by Akaike Information Criterion (AIC) score using Model test 3.7 (Posada & Crandall, 1998). The ML clustering was performed using the Tamura 3-Parameter (Nei & Kumar, 2000; Tamura *et al.,* 2013) and estimates of confidence were based on 1000 bootstrap values. All the sequence gaps involved in the analysis were treated as complete deletion as there were no ambiguous sites.

Population genetic analysis

To obtain a geographical and graphical representation of the variation of the D-loop gene, minimum spanning networks (MSN) of the haplotype (Figure 2) was constructed using the median-joining method in Network v5.0.0.0. (Fluxus Technology 2004-2016) by allowing all the required mutational steps that would eventually link the different sub-networks. The haplogroups were formed based on the results provided by haplotype tree reconstructed using Maximum Likelihood (ML) methods implemented in MEGA v6.0 (Figure 3). The nucleotide diversities (π), the value of the haplotype (h), number of polymorphic sites (S), the mean number of nucleotide differences (K) and nucleotide divergence (Da) were calculated using DnaSP version 5.10.01 (Rozas & Librado, 2009). Nucleotide diversity was calculated to record the level of polymorphism between populations using the mean number of nucleotide difference per site among two populations (Nei & Li, 1979) that was estimated using Jukes & Cantor method (Nei, 1987).

Mantel test was conducted in Arlequin Version 3.5.1.3 (Exoffier *et al.,* 2005) with 1000 permutations to examine the effect of isolation-by-distance (IBD) by testing the correlation between geographical distance and genetic differentiation among populations. The neutrality tests of Tajima's, *D* (Tajima, 1989), Fu & Li's *D** and *F** (Fu & Li, 1993) and Fu's F_s (Fu, 1997) were done in DnaSP v5.10.01. The neutrality tests were used to test the hypothesis that all mutations are selectively neutral (Kimura, 1980). The population expansion in this study was inferred by examining the demographic history of *P. lucasi* using the mismatch distribution analyses implemented in DnaSP version 5.10.01 (Rozas & Librado, 2009). In order to determine the gene flow with an assumption that no mutation has occurred, the level of population subdivision (F_{st}), nucleotide subdivision (N_{st}), and the number of migrants (N*m*) were also calculated using DnaSP version 5.10.01 (Rozas & Librado, 2009). For the analysis of further differentiation among popula-

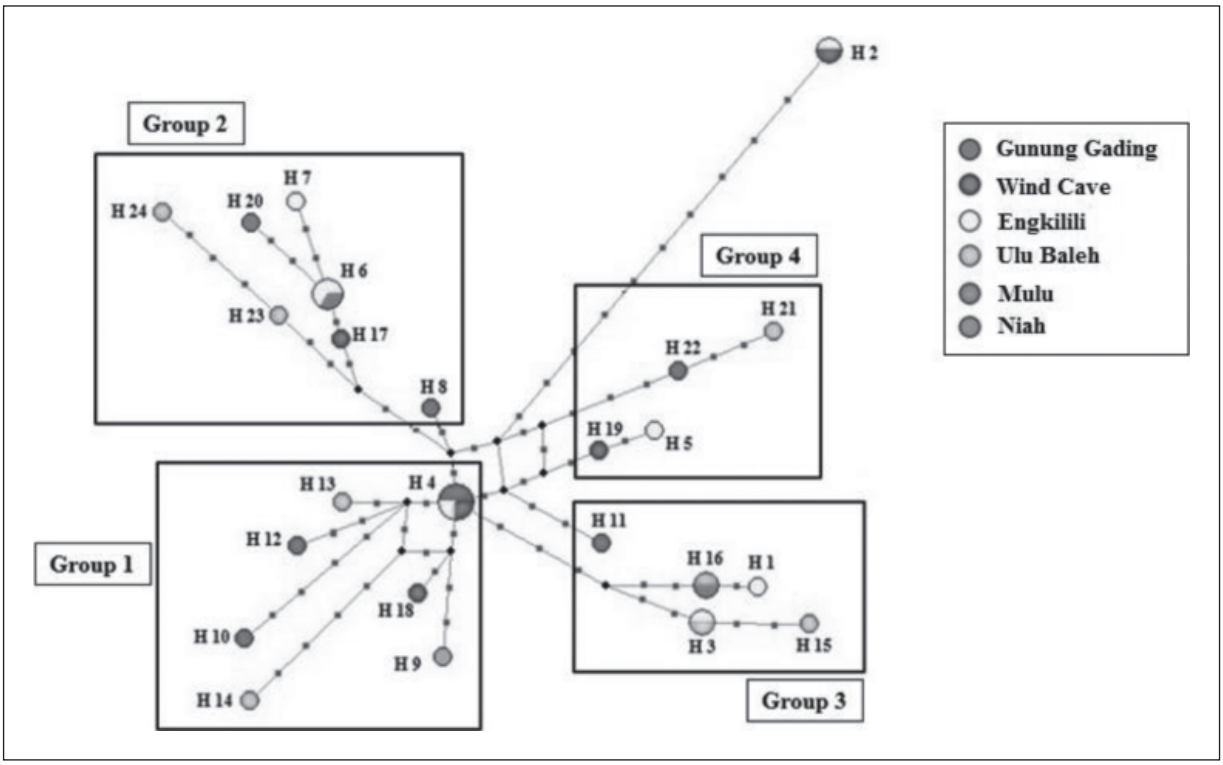

Fig. 2. Haplotype mapping illustrating the 24 assigned haplo-nodes within six localities of *P. lucasi* from Sarawak. Each colour of the nodes represents each locality. The size of the circles is scaled to the haplotype frequency. The number of mutational steps between haplotypes is shown by the red dots lies on the branches that connect the nodes.

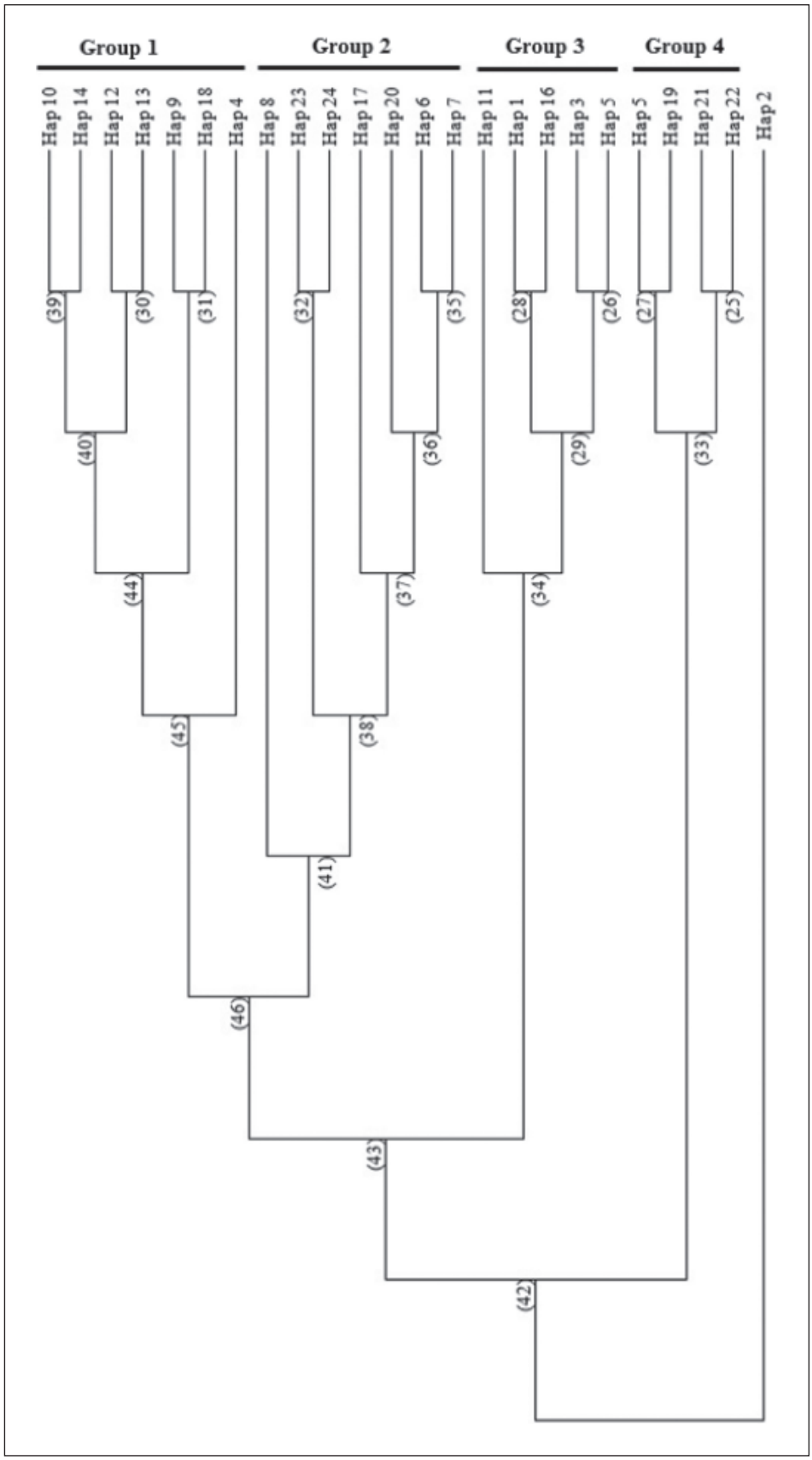

Fig. 3. Maximum likelihood (ML) tree of *P. lucasi* haplotypes in Sarawak referring to 424 bp D-loop mitochondrial gene. Values lie next to the branches are the ML bootstrap approximations with 1000 replicates.

tions, F-statistic (Φ_{st}) (Weir & Cockerham 1984) values were estimated by Analysis of Molecular Variance (AMOVA) with 1000 permutations performed in Arlequin Version 3.5 (Exoffier *et al.,* 2005).

RESULTS

Genetic distance and sequence analysis

A total of 30 sequences with 424 base pairs (bp) in length each without gaps and two sequences with 407 base pairs (bp) in length each with gaps but added with (-) symbol to be 424 bp were aligned successfully for the analysis in this study. From 424 bp sequences, 367 (86.6 %) were conserved sites, 57 (13.4 %) variable sites, 41 (9.67 %) parsimony informative sites and 15 (3.54 %) singleton sites. The mean nucleotide frequencies for thymine (T) = 23.9%, cytosine (C) = 31.9%, adenine (A) = 31.2 and guanine (G) = 13.0%.

The highest genetic distance within localities was recorded for Engkilili (2.35%) and the lowest genetic distance within localities was recorded for Mulu with 1.24% (Table 2). The highest genetic distance among localities (2.32%) was observed between localities of Engkilili and Ulu Baleh while the lowest genetic distance (1.36%) was observed between localities of Mulu and Niah which has the lowest geographical distance (Table 3). Meanwhile, the genetic distance between localities of Gunung Gading and Wind Cave is considered high (2.16%) despite its closest geographical distance (44.6 km; Table 3).

Haplotypes resolution and network analysis

From 32 sequences of six different localities, there are 24 haplotypes with five shared and 19 unique haplotypes (Table 4). Hap 4 has the highest haplotype sample frequencies with Miri (2), Wind Cave (1) and Engkilili (1). Each locality has more than one unique haplotype except for Niah which possesses only one unique haplotype (Hap 9). The localities with the highest number of haplotypes were set by Engkilili and Ulu Baleh with eight haplotypes each (Table 4). There are four haplogroups displaying misclassified localities of *P. lucasi* from all populations: group 1 (Haplotype 4, 9, 10, 12, 13 and 18), group 2 (Hap 6, 7, 8, 17, 20, 23 and 24), group 3 (Hap 1, 3, 15 and 16) and finally group 4 (Hap 5, 19, 21 and 22). Haplotype 2 was not included in the groups as it was not part of any group in the phylogenetic analyses.

Nucleotide divergence among population

A total of 47 segregating sites were detected from 24 haplotypes distributed among six populations of *P. lucasi*. The nucleotide diversity (π) among populations of *P. lucasi* from Sarawak Malaysia ranging from 1.33% to 2.29% with average substitution per rate site (nucleotide divergence, Da) ranging from -0.217% to 0.118% (Table 6). The highest nucleotide diversity was found between Sri Aman and Kapit with the value

Table 2. Genetic distance within six localities of *P. lucasi* in Sarawak.

Locality	Genetic distance (%)	Group	Genetic distance (%)
Engkilili RF	2.35	Sri Aman	2.35
Niah NP	0	Miri	1.27
Mulu NP	1.24		
Ulu Baleh RF	2.29	Kapit	2.29
Gunung Gading NP	2.02	Kuching	2.09
Wind Cave NR	2.02		

Table 3. Genetic distance (%) among localities of P. lucasi in Sarawak (below diagonal) and geographical distance between each locality in km (above diagonal)

Locality	ERF	NNP	MNP	UBRF	GGNP	WCNR
ERF		384.2	485.1	194.1	227.6	187.3
NNP	1.9		126.3	269.1	496.6	484.4
MNP	2.08	1.36		280.4	616.8	602.9
UBRF	2.32	1.82	1.95		518.7	487.1
GGNP	1.98	1.94	2.18	2.25		44.6
WCNR	2.01	1.61	1.71	2.2	2.16	

ERF-Engkilili Reserve Forest, NNP – Niah National Park, MNP – Mulu National Park, UBRF – Ulu Baleh Reserve Forest, GGNP – Gunung Gading, WCNR – Wind Cave National Reserve.

Table 4. List of D-loop haplotypes of *P. lucasi*

Haplotype	ERF	NNP	MNP	UBRF	GGNP	WCNR
Hap 1	1					
Hap 2	1					1
Hap 3	1			1		
Hap 4	1		2			1
Hap 5	1					
Hap 6	2				1	
Hap 7	1					
Hap 8			1			
Hap 9		1				
Hap 10			1			
Hap 11			1			
Hap 12			1			
Hap 13				1		
Hap 14				1		
Hap 15				1		
Hap 16				1	1	
Hap 17						1
Hap 18						1
Hap 19						1
Hap 20					1	
Hap 21				1		
Hap 22			1			
Hap 23				1		
Hap 24				1		
Total	**8**	**1**	**7**	**8**	**3**	**5**

ERF-Engkilili Reserve Forest, NNP – Niah National Park, MNP – Mulu National Park, UBRF – Ulu Baleh Reserve Forest, GGNP – Gunung Gading, WCNR – Wind Cave Nature Reserve.

Table 5. Summary statistics of D-loop mtDNA sequence variation in four populations of *P. lucasi* in Sarawak

Locality/ Population	N	H	S	Hd	Pi	k	*D*	F_s	D*	F*
ERF	8	7	27	0.9643	0.0229	10.5357	-0.7774	-0.359	-0.7933	-0.8798
MNP	7	6	14	0.952	0.0133	5.4286	0.6265	-0.967	-0.4155	-0.5092
NNP	1	1	0	0	0	0	0	0	N/A	N/A
UBRF	8	8	27	1	0.0223	9.0714	-0.6803	-2.385	-0.5551	-0.6508
WCNR	5	5	19	1	0.0226	9.6	-0.9685	-0.393	-0.9685	-1.04
GGNP	3	3	12	1	0.0204	0	0	0	N/A	N/A
Sri Aman	8	7	27	0.9643	0.0229	10.5357	-0.7774	-0.359	-0.7933	-0.8798
Miri	8	7	16	0.9643	0.0125	5.5	-0.8312	-1.688	-0.5257	-0.6636
Kapit	8	8	27	1	0.0223	9.0714	-0.6803	-2.385	-0.5551	-0.6508
Kuching	8	8	27	1	0.0204	9.5357	-1.2079	-2.276	-1.3686	-1.4831
Total	**32**	**24**	**47**	**0.9758**	**0.0233**	**7.9597**	**-1.1689**	**-10.315**	**-0.1452**	**-0.5746**

N – the number of sequences analyzed; H – the number of haplotypes; S – segregating sites; Hd haplotype diversity; Pi – nucleotide diversity; k – nucleotide differences; D – Tajima's statistic; Fs-Fu's statistic; D* and F* – Fu and Li's statistic. P > 0.1 – the significance of neutrality test was determined using coalescent simulation in DnaSP v5.10.01 (Librado and Rozas, 2009). N/A – Data not available. None of the statistical results are significant.

of 2.262% with nucleotide differences of 9.18 (Table 6). The lowest nucleotide diversity was 1.736% between Miri and Kuching with nucleotide divergence of 0.101% (Table 6). Mantel test showed that there is a significant relationship between nucleotide divergence and geographic distance (correlation coefficient, r = 0.7765, significant, P = 0.041) among populations of *P. lucasi* (Table 7).

Population expansion and neutrality test

Populations of *P. lucasi* from Kapit and Kuching had the highest haplotype diversity with

Table 6. Comparison data statistic between four population groupings of *P. lucasi* in Sarawak

Population	Distance (km)	Gd (%)	Pi	Da	k	Nm	Fst	Nst
Sri Aman - Miri	282.67	1.93	0.01906	0.00118	7.758	4.73	0.06239	0.06174
Sri Aman - Kapit	194.14	2.32	0.02262	0.00002	9.183	6.95	0.00097	0.0011
Sri Aman - Kuching	157.09	1.84	0.02241	-0.00217	9.5	35.13	-0.11124	-0.11161
Miri - Kapit	109.28	2.00	0.01773	0.00059	7.2	5.5	0.03297	0.03212
Miri - Kuching	348.23	1.75	0.01736	0.00071	7.067	5.08	0.04164	0.04148
Kapit - Kuching	291.58	2.22	0.02151	0.00025	8.733	6.39	0.01169	0.01179

Gd–Genetic distance; Pi–nucleotide diversity; Da–Net nucleotide divergence; k–nucleotide differences; Nm–Gene Flow; Fst–Population subdivision; Nst–Nucleotide subdivision.

Table 7. Measures of geographical population differentiation in *P. lucasi* based on analysis of molecular variance (AMOVA)

Source of variation	Variance components	Percentage of variation	Fixation index (Φ)	Significant test (P)
Among population	0.09063	1.91	Φ_{ct} = 0.01914	0.34115
Among localities between populations	-0.09172	-1.94	Φ_{sc} = -0.01975	0.31085
Within localities	4.73677	100.02	Φ_{st} = -0.00023	0.5044

*Significant P < 0.05.

(h = 1.000). This was followed by Sri Aman (h = 0.9643) and Miri (h = 0.9520) populations with slightly lower value (Table 5). Niah has the lowest haplotype diversity for (h = 0.0000) with no segregating sites as only one sequence was analyzed from that locality. Tajima's *D*, Fu's F_s and Fu and Li's *D** and *F** statistics were used to test the presence of demographic expansion. Tajima's *D* analyses for all populations showed negative values with a total Tajima's D value (-1.2732; Table 5). The negative value was observed for all populations tested with Fu's, F_s and Fu and Li's, *D** and *F** indicating the existence of rare haplotypes (Fu, 1997).

The mismatch distribution of pairwise nucleotide differences among D-loop sequences revealed multimodal distribution characteristic (Figure 4a). This showed the observed distribution deviated extremely from the expected population expansion model which indicates that the population might experience long-term demographic stability (Sherry *et al.,* 1994; Landry & Bernatchez, 2001; Liu *et al.,* 2006). The observed allelic frequency spectrum was parallel to the expected allelic frequencies under the null hypothesis of no population change (Figure 4b).

Population subdivision

AMOVA analysis has resulted in 100% of the variation within localities and 1.91% of the variation among the population. However, none of that data obtained above were statistically significant (Table 7). The lowest gene flow (N*m*= 4.73) with the highest population structuring (F_{st} = 0.05714) was found between the populations of Sri Aman and Miri (Table 6). In contrast, a very high gene flow (N*m*= 35.13) was found between Sri Aman and Kuching with negative values of F_{st} (-0.11124) and N_{st} (-0.11161) (Table 6).

DISCUSSION

Overall, a high level of haplotype diversity and a low level of nucleotide diversity were recorded from sequence analysis of *P. lucasi* populations in Sarawak. All the haplotypes from Miri were considered unique except one (haplotype 4) which was shared by the population of Sri Aman (Engkilili), Miri (Mulu) and Kuching (Wind Cave). This is comparable to the study conducted by Mohd-Ridwan and Abdullah (2012), where the formation of haplogroups and sharing of haplotypes between Miri and Kuching populations indicate that these two populations could be the source of refugium for *P. lucasi* during Pleistocene. Out of 24 haplotypes analyzed in this study, only five haplotypes were shared. Lack of shared haplotypes is often due to large geographical distances that might become barriers for gene flow, resulting in unique haplotypes limited to single locality (Whitlock & McCauley, 1999). MSN analysis showed many unresolved relationships among haplotypes and this is possibly due to the low bootstrap values in

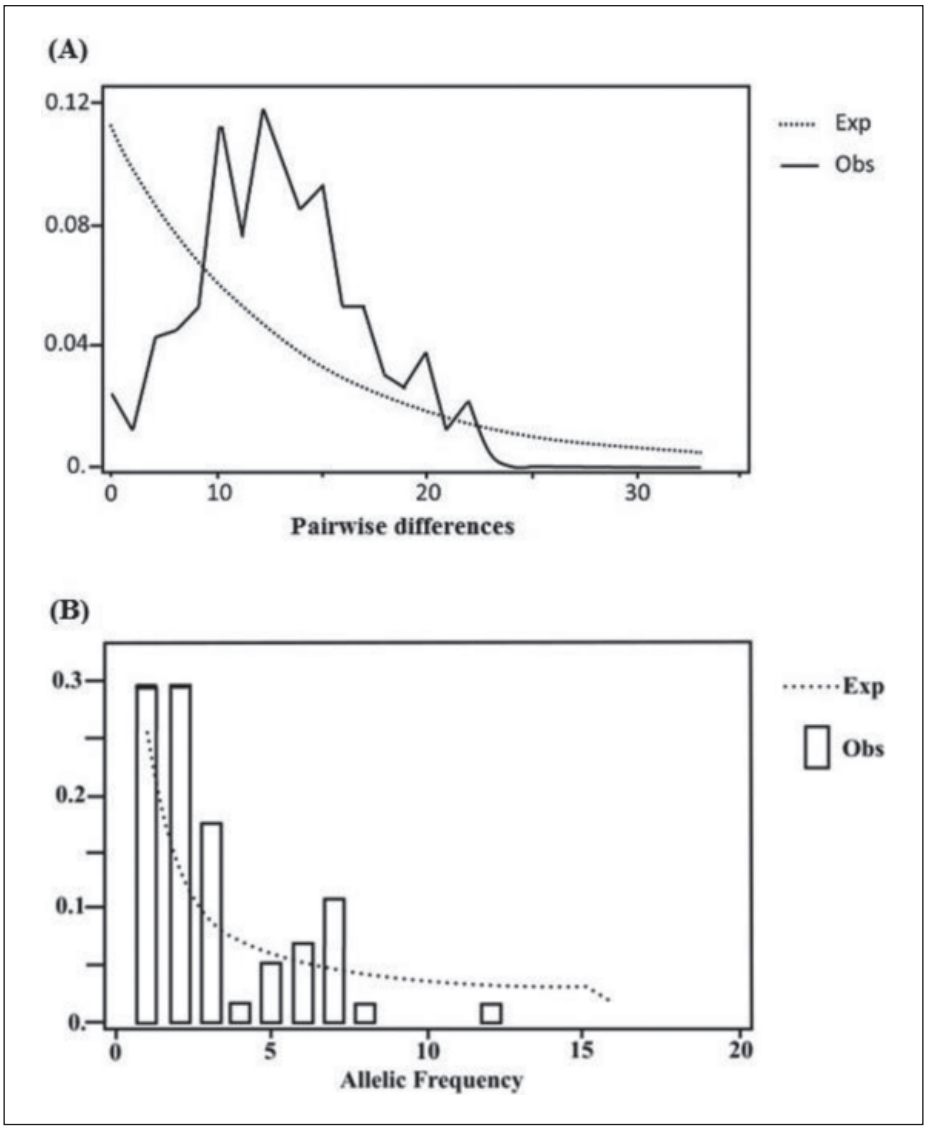

Fig. 4. Population expansion models of D-loop mitochondrial gene of *P. lucasi*. (A) Mismatch distribution of pairwise nucleotide differences with multimodal distribution and (B) Allele frequency spectrum with no deviation between observed and expected frequencies of segregating sites among six populations of *P. lucasi* in Sarawak Malaysia under the null hypothesis of no population change. (Exp-Expected; Obs-Observed).

haplotype tree and lack of sample coverage (Templeton & Sing, 1993; Brown *et al.,* 2011). Moreover, haplotypes misclassification of *P. lucasi* seen in every haplogroup is probably due to its ability to conduct long-distance flight for foraging. Very similar reasoning was given to the misclassification of nectarivorous bats into various geographical clades in Malaysia (Jayaraj, 2008).

The moderate level of genetic variation within the population of *P. lucasi* showed that this population might be recovering from recent catastrophic incidents. Habitat disturbance and climate change are also responsible for the reduction in genetic variability of this population (Mohd-Ridwan & Abdullah, 2012). Major disturbance of caves in Borneo such as ungovernable guano mining, ecotourism, and swift nest collection would threaten the long-term survival of cave bats (Mohd-Ridwan & Abdullah, 2012). According to Storz (2002), the environment of the surrounding landscape can affect the genetic variation of a species. However, in another study comparing the interspecific genetic diversity of two bats species showed no significant genetic diversity recorded despite the environmental differences at the habitats of each bat species (Asher, 2009). They suggested that these were probably because of the population sizes of the bats do not vary significantly between the localities and also the high level of gene flow within the populations.

Gene flow can be influenced by the rate and distance of dispersal events as it is expected to increase with the increasing distance between populations or vice versa (Flanders *et al.,* 2009; Mohd-Ridwan & Abdullah, 2012). In this study, all populations have moderate to high level of gene flow. The highest gene flow (35.13) was observed between populations of Sri Aman and Kuching with the negative and lowest value of F_{st} (-0.11124). This corresponds to its lowest geographical distance (109.3 km) that may have facilitated the high migration rate. On the other hand, populations from Sri Aman and Miri have the lowest gene flow (4.73) with the highest value of F_{st} (0.06239) indicating strong population structuring with large geographical distance (second largest distance between studied population). The 19 unique haplotypes found in this study could be the result of isolated populations or populations that have fewer gene flows due to geographical barriers (Aboim *et al.,* 2005). Given that our study showed that overall rate of migration (N*m*) studied here are > 1, then gene flow can be considered as the main source of genetic variation in this species (Li, 2007).

A moderate level of genetic variation found in this study reflects a population expansion after a low effective population size (Aboim *et al.,* 2005). Such population might be experiencing bottleneck effect or recent demographic isolation but capable of recovering from those catastrophic events (Avise, 2000). However, the demographic analysis of this species showed a ragged and multimodal graph for all mismatch distribution reflecting that the population is undergoing long-term demographic stability (Campbell *et al.,* 2006; Lloyd, 2003). This is further supported by allelic frequency histogram where the observed shape is parallel to the expected curve, indicating that there is no occurrence of population change. A similar pattern was also observed in other fruit bats from the genus *Cynopterus* (Campbell *et al.,* 2006). The stable population trend observed here may be influenced by their ability to fly to explore a new area and different feeding niches (Francis, 2008). Moreover, the low percentage of genetic distances among and within populations could account for the high gene flow among *P. lucasi* in Sarawak.

Mantel test showed that there is no relationship between geographic distance and nucleotide divergence as none of the data in AMOVA analysis are significant ($P < 0.05$). These might suggest that nucleotide divergence values of *P. lucasi* were not structured by the geographical distances between studied populations. This finding is similar to Mohd-Ridwan & Abdullah (2012) but their study showed that there is the high genetic distance (K2P = ~ 5%) between Miri and Kuching population suggesting the existence of unrecognized lineage within *P. lucasi* complex. Despite broader sampling coverage and use of fast evolving gene, our study only documented the highest divergence at 2.32% (between Sri Aman and Kapit). It is possible that we do not capture the variation due to the low sample size per site compared to the previous study. Our finding along with high gene flow observed between *P. lucasi* population in Sarawak support the panmixia population observed here.

CONCLUSION

In conclusion, moderate level of genetic variation is found among the population of *P. lucasi* in this study. The high gene flow and low population subdivision values are reflected in low population structuring between populations of *P. lucasi* in Sarawak. The high level of gene flow in this study showed that *P. lucasi* can travel far from their roosting sites and indirectly contribute towards forest ecosystem through pollination and seed dispersal. There is no species complexity detected in this study as the genetic divergence among and within populations are low (<5%). Information from Sabah and Kalimantan may provide important insight into the population history of this species in Borneo that may not be captured by our samples.

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