Mitochondrial DNA Variability of Edible Bird Nest Swiftlet (*Aerodramus Fuciphagus*) Colonies In Malaysia.

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Abstract- Edible Bird Nest Swiftlets naturally inhabits the lime stone caves of South East Asia, but recently swiftlet ranching in house farms has become popular. These swiftlets are of great commercial importance because of their unique nest produced wholly from the pair of salivary gland and highly valued for their nutritional, medicinal and aphrodisiac properties. The study elucidates mitochondrial variability information of *A. fuciphagus* by analysing two mtDNA genes (*cytb* and *ND5*). For the mtDNA analysis, DNA samples, extracted from tissue, blood and nests of 28 EBN swiftlets from six locations (Sungkai in Perak, Kuala Terengganu in Terengganu, Tanah Merah in Kelantan, Kota Tinggi in Johor, Gua Madai and Pitas in Sabah) were used. The DNA was subjected to PCR amplification of two mtDNA gene loci namely *cytb* and *ND5*. *Cytb* had the highest variation with eight haplotypes while *ND5* had five. The neutrality tests were negative values for the Tajuma's D and Fu and Li's D. The shared haplotypes indicate high gene flow between locations with two separable groups that are genetically different.

Index Terms – Edible Bird Nest swiftlet, Mitochondrial DNA, Genetic variability, Colonies.

1. INTRODUCTION

Edible Bird Nest (EBN) Swiftlets are small sized insectivorous swifts from the family Apodidae that inhabit limestone caves of many part of South East Asia (Chantler et al., 1999, Marcone, 2005). They are first class flyers flying at high velocity (Videler et al, 2004) and possess the ability to find their way in deep darkness through echolocation, an advantage that make them free visually orienting predators (Fenton, 1975). They are diurnal and have good sight for capturing flying insects during broad daylight (Brinkløvet et.al, 2013). Swiflets produce edible nest which is highly sought after delicacy among Chinese gourmands.

EBN swiftlet build nest wholly of its secretion from the pair of sublingual salivary glands, constructed without moss, feather or twigs (Marshall and Folley, 2007). Harvesting of EBN from natural wide colonies is dated back to the late sixteenth century by the Chinese society (Lim and Cranbrook, 2002).

EBNs are highly sought after due to their nutritional, aphrodisiac and purported health benefits (Norhayatti et al, 2010, Guo et al, 2006) which includes possessing useful properties that tone up the organs of the body, relieve gastric problems and aids kidney function (Marcone, 2005). The nest is among the most expensive animal product in the world and because of this, there has been great exploitation across the regions (Sankaran, 2001). The local EBN industry has been domesticating these economically important birds there by creating a decline in natural cave populations (Aowphol et al, 2008). Recently, there has been an expansion in the EBN swiftlets house farms due to the construction of numerous large buildings for the purpose of housing large colonies of the birds; this has led to an increased number of birds but may have an adverse effect on the overall population of swiftlets (Chantler and Driiessens, 2000, Aowphol et al., 2008). *Aerodramus fuciphagus* have a high degree of nest site fidelity (Nugroho and Whendrato, 1996; Lim and Cranbrook, 2014) and are monogamous breeder (Viruhpintu et al., 2002). Although some growers observed that some other species such as glossy swiftlet (*Collocalia esculenta*), Mossy nest swiftlet (*Aerodramus salangana*) and cave swiftlet (*C. linchii*) are good foster parents and would hatch and rear chicks from eggs of manmade swiftlet farms when transferred into their nest; this act has led to fast establishment of new colonies of EBN swiftlet (Thornburn, 2015). Special consideration has to be given to EBN swiftlet being unique specie and the attempt to increase their numbers, maintaining their genetic stability of the populations is paramount.

Mithochondrial DNA (mtDNA) has been widely used in animal phylogenetic studies because it evolves rapidly more than nuclear DNA, leading to accumulation of differences between closely related species (Mindell et al., 1997). MtDNA divergence have been observed in sister species and more generally ''biotic entities registered in mtDNA genealogies and traditional taxonomic assignment tends to converge'' (Avise & Walker, 1999). The general concordance of mtDNA trees with specie trees means that

analysing their mtDNA can identify species of specimen (Herbert et al, 2004). Although many species show phylogeographic subdivisions usually uniting into single lineages, sequence divergences are greater among species than within species and thus mtDNA genealogies captures biological discontinuities recognized by taxonomist as species.

Cytochrome b gene (*cytb*) is the most frequently used mtDNA marker (Parson et al, 2000; Burns et al, 2009) and most extensively sequenced gene to date for vertebrates (Irwin et al, 1991). *Cytb* gene is rich in phylogenetic information and there is better characterization of the molecular systems evolutionary dynamics (Esposti et al, 1993). NADH dehydrogenase subunit (*ND5*) genes have been used for avian specie identification and phylogenetic reconstruction based on fewer anomalies (Nakamura, 2009), relative similarities among lineage nucleotide composition and phylogenetic utility in distantly and closely related lineages (Paton and Baker, 2006). Adequate knowledge about the delineation among swiftlet specie in Malaysia is necessary to evaluate genetic variation and its effect on the growing industry on local swiftlet populations. The study aimed to assess mtDNA genes (*cytb* and *ND5*) and elucidate the genetic variability of *A. fuciphagus* across locations in Malaysia.

2. MATERIAL AND METHOD

SAMPLING

Blood samples of EBN swiftlets were collected from house farms at Sungkai Perak (five individual); Tanah Merah, Kelantan (five individuals). Tissues (Dead birds) were collected from house farms at Kuala Terengganu, Terengganu (five individual). Edible nest from house farms at Kota Tinggi, Johor (five), Pitas Sabah (three) and nest from cave at Gua Madai, Sabah (five). Three Pacific swallows (*Hirundo tahitica*) were collected from Sungkai, Perak to serve as an out-group for comparison.

DNA EXTRACTION

Genomic DNA was extracted from blood and tissue samples using the DNeasy blood and tissue kit (Qiagen, California) following the manufacturer's instructions. DNA extraction from the nest was done using the wizard extraction protocol. Two hundred and fifty milligram of dried EBN was weighed and crushed. The powdered samples was placed into 15ml falcon tube and 3ml of extraction buffer (150mM NaCl, 2mM EDTA, 1% SDS (w/v), 10 mM Tris: HCl pH 8.0) was added and mixed. This was followed by addition of 350ml guanidine thiocyanate (Sigma, USA) and 140µl of Proteinase K (>600 mAU/ml). The mixture was incubated at 56ºC for 3 h in a water bath. The supernatant was transferred into 2ml micro centrifuge tube and Wizard DNA Clean- Up Resin (Promega USA) was used following the manufacturer's instruction.

PCR AMPLIFICATION AND SEQUENCING

The *cytb* and *ND5* regions of the mtDNA were amplified using the primers cyt-*b*258 (5'-CGGAGCCTCATTCTTCTTCA-3') and cyt-*b*820 (5'-ATCATTCCGGCTTGATATGG-3') for *cytb*, designed by Aowphol et al, (2008) and F: (5;-CCTATTTTGCGGATGTCTTGTTC-3'), R: (5'- CAGGAAAATCCGCTCAATTCGG) for *ND5* (Garcia-Moreno et al, 1999). PCR amplification was carried out in 30 µL reactions containing 3 µL of 10X Pfu Buffer with MgSO4, 0.6 µL (10 mM) dNTP, 0.3 µL (2.5U) of Pfu DNA polymerase, 0.1 µM of each primer and 50 ng of DNA template. The PCR protocol for the amplification of the *cytb* region consisted of an initial denaturisation for 5 min at 95°C, followed by 8 cycles of denaturation at 95°C for 30 s, annealing at 64°C for 30 s, extension at 72°C for 30 s, followed by 29 cycles of denaturation at 95°C for 30s, annealing at 54°C for 30s, extension for 1min at 72°C and a final extension for 10 min at 72°C. The PCR protocol for the amplification of the *ND5* region consisted of an initial denaturisation for 5 min at 95°C, followed by 35 cycles of denaturation at 94°C for 1min, annealing at 64°C for 30 s, extension at 72°C for 30 s, followed by 29 cycles of denaturation at 95°C for 30s, annealing at 50°C for 1min, extension at 72°C for 1min and a final extension for 10 min at 72°C. The PCR products were run in 1.5% low melt agarose gel with ethidium bromide and the gel were viewed under ultraviolent light. Gels were cut and purified using the GeneAll gel purification kit (Korea). Cloning of mtDNA regions was performed using Thermo Scientific CloneJET® PCR cloning kit (Germany). 3 colonies were picked from each sample. Plasmid extraction was done using GeneAll® HYBRID-Q™ PLASMID RAPIDPREP (Korea). The purified plasmids were sequenced using the service provider (First Base Sdn. Bhd., Malaysia).

MtDNA SEQUENCE ANALYSIS

The DNA sequences obtained from the clones were subjected to multiple alignments using BioEDit v7.0.9 software and consensus sequences were generated. Phylogenetic analyses were performed with the aligned sequences using MEGA5 software (Tamura et al., 2011). Best Modal Test (MEGA5) for each gene was determined by picking the lowest Bayesian Identity Criterion (BIC). Jukes-Cantor model was used to test the suitability of the data set for Neighbour Joining (NJ) trees, if average pairwise distance is <1.0, then it is applicable. Calculation of the distance values among mtDNA fragments sequences were done using Tamura – Nei 3 Parameter model based on the BIC value implemented in MEGA5. Performing the bootstrap test of phylogeny, bootstrap replicates of 1000 and the default random seed were used. The haplotype and nucleotide diversity were done using DnaSP version 3 (Rozas and Rozas, 1999). Additional samples of mtDNA fragments of other species were obtained from GenBank for comparison with EBN swiftlets samples used in this study.

3. RESULTS AND DISCUSSION

Aligned DNA sequences of *cytb* region (563bp) revealed a total of 14 polymorphic sites and 8 haplotypes (Tab 1). Among the 28 samples used, haplotype 1 was the most frequent, exhibited by 13 individuals across four locations. The out-group *H. tahitica* also exhibited this haplotype meaning homology is observed in both species. Genetic diversity of mtDNA *cytb* (Tab 2) shows the overall haplotype and nucleotide diversity to be 0.847 and 0.009 respectively. There were no significant differences (P>0.10) in the neutrality test based on the negative value of Tajuma's D and Fu & Li's D (P>0.10). Neighbour Joining tree (Fig 1) constructed shows there were sub- clades with majority of the sequences being tightly associated. Sungkai, Perak and Tanah Merah samples showed close genetic affinities from evidence on the branching pattern. There are no clear evidences of geographical clustering among EBN swiftlets populations. Haplotype 2-8 were in sub- clade separated from *A. fuciphagus amechanus spp*. The DNA sequences have been deposited in GenBank under accession number KJ671364- KJ671391.

A fragment of (418bp) was derived for EBN swiftlets from the amplification of mtDNA *ND5*. Six polymorphic sites and five haplotypes were detected (Tab 3). Haplotype 1 was common to all location. Samples from Kota Tinggi and Sungkai had two unique haplotypes each. There were no significant differences based on the negative Tajuma's D and Fu & Li's D (P>0.10) (Tab 4). Negative values are indicative of a possible departure from neutral expectations. Neighbour joining constructed shows no clear evidences of geographical clustering among population. Haplotypes were grouped into a clade because there were no reference sequences of *A.fuciphagus* for comparison in the GenBank (Fig 2). DNA sequences have been deposited in GenBank under accession number KJ671392-KJ671419. Intraspecific variations observed in *ND5* are due to the slow evolving nature of the gene (Mindell and Honeycutt, 1990).

In *cytb*, the most frequent haplotype were found in samples representing both cave and house population from South of Peninsular Malaysia (Kota Tinggi), East of Peninsular Malaysia (Kuala Terengganu) and East Malaysia (Gua Madai and Pitas) showing a substantial mtDNA gene flow and structured demographic population. These regions of mtDNA have been used to detect structure of populations in many avian species because it evolves relatively fast (Buer-kle 1999; Burg and Croxall, 2001). House samples from East Peninsular (Tanah Merah) and North Peninsular (Sungkai) had many variations but with fewer frequency of haplotypes. Majority of the haplotypes (H2 – H8) from the *cytb* are less abundant, less widespread and did not cluster with samples retrieved from the GenBank. The most frequent haplotype was unique suggesting significant genetic distinction and there may be sub-species delineation in the swiftlet population of Malaysia. Phylogenetic tree show that EBN swiftlet form a moderately supported cluster with brown- rump swiftlet *A.fuciphagus vestitus* (BS 79%) and they are different from *A. fuciphagus amechanus* spp. This was supported by Cranbrook et al, 2013, showing that the name *A. fuciphagus amechanus* is not applicable to swiftlet from Peninsular Malaysia. Similar findings of two clades populations were observed in the work of (Goh et al, 2018) among house farm swiflets in Malaysia, the first clade was samples from across Peninsular Malaysia and Borneo States of Malaysia while the second clade was from Pahang, Malaysia. This was also justified in the conclusion by (Chasen, 1933, 1935) that there are two *Aerodramus* swiftlet making white nests and they are separated by habits which include choice of nesting site.

The Neutrality test values were negative for the gene loci indicating possible departure from neutral expectation. It could be that the population has not yet reached equilibrium with most mutation tending to be unique to a single lineage or that the sample sizes were small. However, negative values were also reported in larger samples in Thailand, indicating high genetic variation within colonies than between colonies (Aowphol et al, 2008).

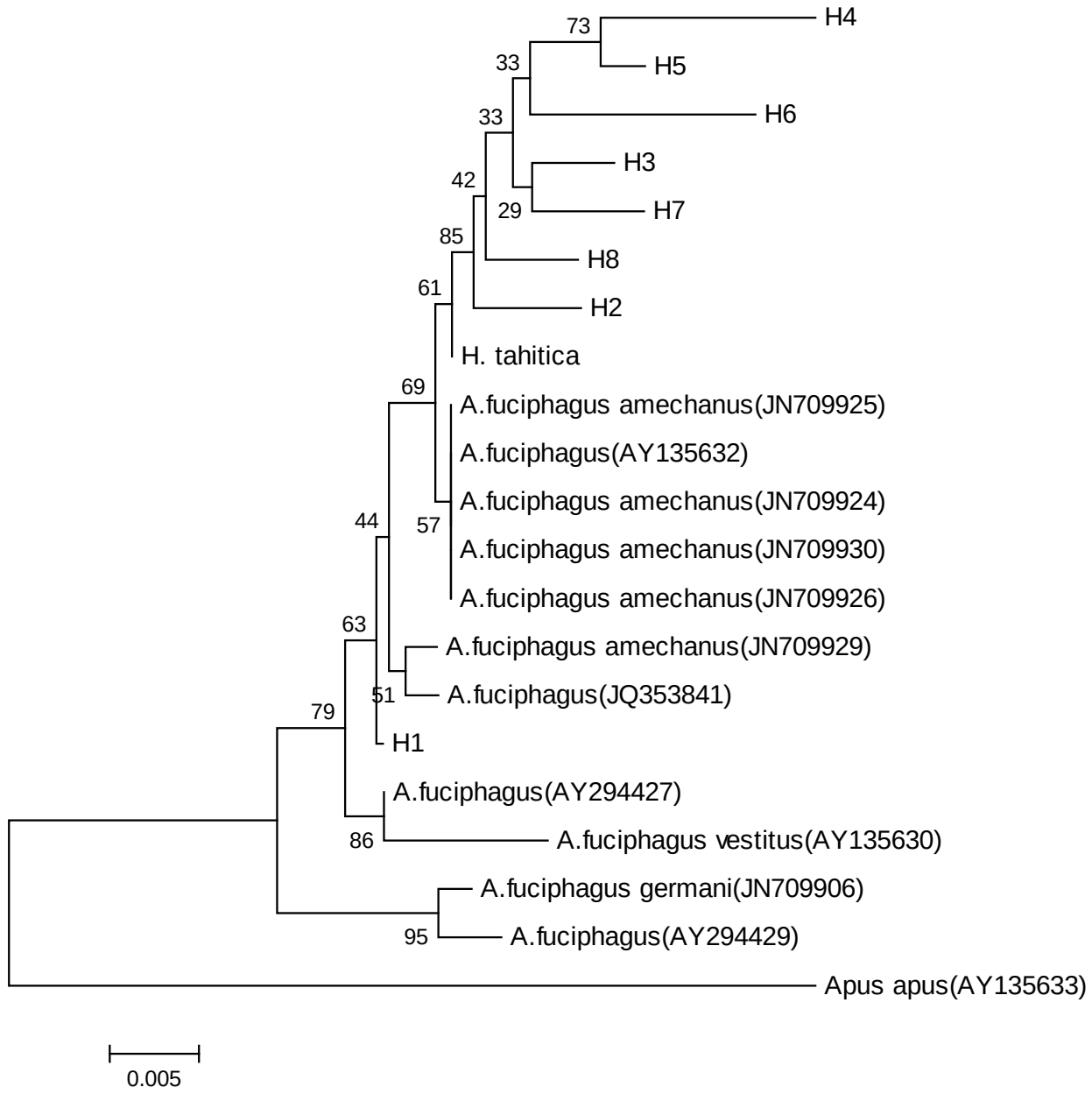

Fig 1. Neighbour Joining phylogenetic trees based on the haplotypes of mtDNA *cytb* locus in *A. fuciphagus.* Haplotypes labelled as *A. fuciphagus* are those retrieved from the GenBank. *Apus apus* was used as an outgroup because *H. tahitica* had similar haplotype sequence as *A. fuciphagus.* Numbers indicate the percentage of confidence level for each node estimated at 1000 bootstrap value.

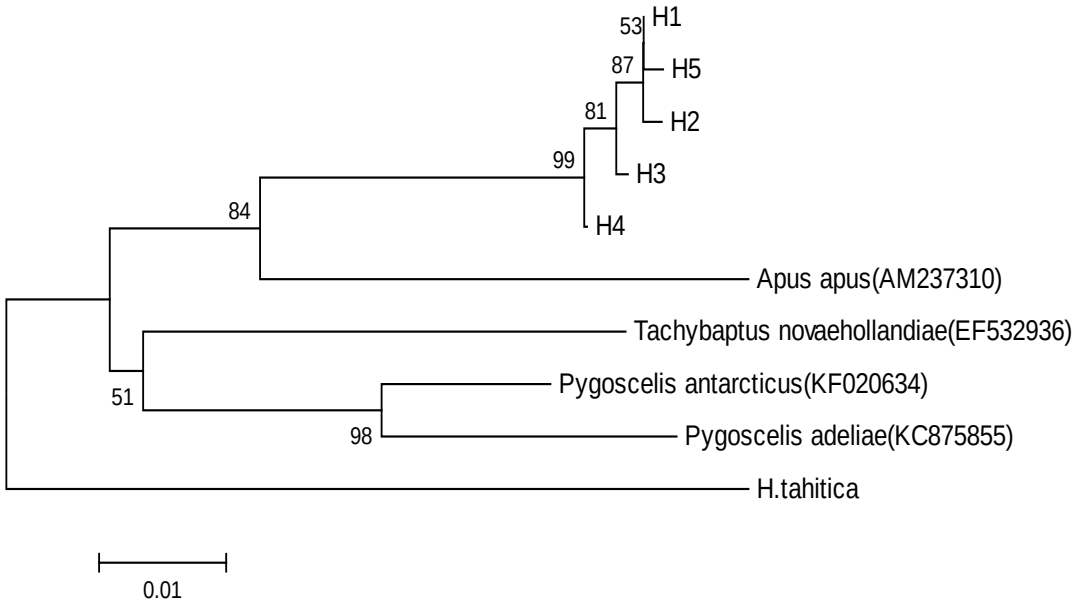

Fig 2. Neighbour Joining phylogenetic trees based on the mtDNA *ND5* gene locus of five haplotypes used in this study and other sequence retrieved from GenBank. *H. tahitica* was used as outgroup. Numbers indicate the Percentage of confidence level for each node estimated at 1000 bootstrap value.

Genetic variation is influenced by a lot of factors such as reproduction, behaviour, migration, population size, natural selection and historical events. There are no natural inland population of white nest swiftlet in Peninsular Malaysia and despite the high number of house farms, EBN swiftlets usually do not revert to cave nesting site (Goh et al, 2018). But with the availability of improved environment and reproductive propensity by man, EBN swiftlets has increased in both number of individuals and the colonies. Initially, they are sourced from natural caves and house farms buildings but more recently, birds in the new buildings may have come from any established colonies in buildings. After earlier population separated from their natural habitat, there was continuity of populations of house farms resulting in homogeneity of their sequence thereby making them to be distinct. EBN swiftlets have high level nest site fidelity which could have led to genetic differentiation but it was not observed in this study. We found lack of genetic structure for EBN swiftlets of Malaysia but both clade (H1 and H2- H8) observed are genetically separable. The result of this study could be used as information to monitor the changes in the genetic variability of the local EBN swiftlet population. Genetic study should not be limited to mtDNA loci, nuclear markers such as microsatellites and SNPs in nuclear DNA should also be used.

Table 1. Haplotypes showing the polymorphic sites, base polymorphism and distribution across locations for mtDNA *cytb* region

	Position														Frequency							
Haplotype	12 4	51 3	51 4	51 9	52 3	52 5	52 7	52 9	53 0	53 1	53 2	53 3	53 4	53 5	SP	TM	KT	GM	PT	KG	Total No of samples	% of total no of haplotypes
1	T	T	C	C	C	A	C	C	C	T	A	G	T	A			5	3	1	4	13	46.4
2	C	.	.	.	.	.	.	.	.	.	.	.	.	.				2	2	1	5	17.9
3	.	.	.	T	.	.	.	.	.	G	T	.	C	C	1	1					2	7.1
4	.	.	.	.	G	G	T	.	T	.	.	A	C	.	1	1					2	7.1
5	.	.	.	.	.	C	.	.	T	.	.	C	C	C	1	1					2	7.1
6	.	.	.	.	.	C	.	.	T	.	.	C	.	C	1	1					2	7.1
7	T	.	.	T	.	C	.	A	A	C	C	.	.	.	1						1	3.6
8	C	.	.	T	.	C	.	.	.	.	.	.	.	.	.	1					1	3.6
Total No of Haplotypes															5	5	1	2	2	2	28	100
H. tahitica	.	.	.	.	.	.	.	.	.	.	.	.	.	.							3	

SP- Sungkai, TM- Tanah Merah, GM- Gua Madai and KT- Kuala Terengganu, PT- Pitas, KG- Kota Tinggi. A – adenine, C – cytosine, G- guanine, T – Thymine.
Position numbering based on the sequences generated in this study.

Table 2. Genetic diversity indices of the mtDNA *cytb* region in six populations across Malaysia.

Population	**N**	**Polymorphic site**	**No of haplotype**	**Haplotype Diversity (H)**	**Nucleotide Diversity (π)**	**Tajima's D**	**Fu and Li's D**
Sungkai	5	14	5	1.000	0.017	-0.884	-0.253
Kuala Terengganu	5	5	1	0.700	0.004	-1.124	-1.124
Gua Madai	5	3	2	0.900	0.001	-0.175	-0.175
Tanah Merah	5	14	5	1.000	0.017	-0.340	-0.065
Pitas	3	1	2	0.667	0.001	-	-
Kota Tinggi	5	1	2	0.440	0.001	-0.817	-0.817
Total	**28**	**14**	**8**	**0.847**	**0.009**	**-1.522**	**-1.262**

Table 3. Haplotypes showing the polymorphic sites, base polymorphism and distribution across location in mtDNA *ND5* region

	Position						Frequency							
Haplotype	0 4 1	1 0 2	2 0 2	3 2 6	3 2 9	3 3 2	SP	TM	KT	GM	PT	KG	Total No of samples	% of total no of haplotypes
1	T	C	G	T	C	C	3	5	5	5	3	3	24	85.7
2	.	T	.	.	.	.	1						1	3.6
3	C	.	.	C	.	.	1						1	3.6
4	.	.	.	C	T	A						1	1	3.6
5	.	.	A	.	.	.						1	1	3.6
Total No of Haplotypes							3	1	1	1	1	3	28	100
H. tahitica	.	.	.	C	T	A							3	

SP- Sungkai, TM- Tanah Merah, GM- Gua Madai and KT- Kuala Terengganu, PT- Pitas, KG- Kota Tinggi. A – adenine, C – cytosine, G- guanine, T – thymine. *H. tahitica* – outgroup. Position numbering based on the sequences generated in this study.

Table 4. Genetic diversity indices for the MtDNA *ND5* region in six populations across Malaysia

Population	**N**	**Polymorphic site**	**No of haplotype**	**Haplotype Diversity (H)**	**Nucleotide Diversity(π)**	**Tajima's D**	**Fu and Li's D**
Sungkai	5	3	3	0.700	0.003	-1.048	-1.048
Kuala Terengganu	5	0	1	0.000		-	-
Gua Madai	5	0	1	0.000		-	-
Tanah Merah	5	0	1	0.000		-	-
Pitas	3	0	1	0.000		-	-
Kota Tinggi	5	4	3	0.700	0.004	-1.094	-1.094
Total	**28**	**6**	**5**	**0.270**	**0.001**	**-1.972**	**-2.595**

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Reference

[1] Aowphol, A, Voris, H. K, Feldheim, K. A, Harnyuttanakorn, P., and Thirakhupt, K. *Genetic Homogeneity Among Colonies of the White-Nest Swiftlet (Aerodramus fuciphagus) in Thailand*. Zoological Science, 2008, 25(4), 372-380.

[2] Avise, J. C, Walker, D. *Species realities and numbers in sexual vertebrates: perspectives from an asexually transmitted genome*. Proceedings of the National Academy of Sciences, 1999, 96(3), 992-995.

[3] Brinkløv, S, Fenton, M.B and Radcliffe, J.M. *Echolocation in Oilbirds and Swiftlets*. Frontiers in Physiology 2013, 4(123), 1-12.

[4] Buerkle, C. A. *The historical pattern of gene flow among migratory and non-migratory populations of prairie warblers Aves: Parulinae*. Evolution, 1999, 53, 1915-1924.

[5] Burg, T. M and Croxall, J. P. *Global relationships amongst black-browed and grey-headed albatrosses: analysis of population structure using mitochondrial DNA and microsatellites*. Molecular Ecology, 2001, 10(11), 2647-2660.

[6] Burns, K. J, Racicot and R. A. *Molecular phylogenetics of a clade of lowland tanagers: implications for avian participation in the Great American Interchange*. The Auk, 2009, 126(3), 635-648.

[7] Chantler, P and Driessens, G. *Swifts: A Guide to the Swifts and Treeswifts of the World*. A&C Black, 2000.

[8] Chantler, P, Wells, D and Schuchmann, K. *Family Apodidae (Swifts).* Handbook of the Birds of the World, 1999, 5, 388-457.
[9] Chasen F.N. *Report on the Birds' Nest Caves and Industry of British North Borneo with special Reference to the Gomanton Caves, Jesselton*, Government Printer,1931, 24.
[10] Chasen F.N. *A handlist of Malaysia birds*. Bulletin of the Raffles Museum, 1935, 11: i-xx, 1-389.
[11] Cranbrook, E. O, Lim, G. W, Koon, L. C and Rahman, M. A. *The species of white-nest swiftlets (Apodidae, Collocaliini) of Malaysia and the origins of house-farm birds: morphometric and genetic evidence.* Forktail, 2013, (29), 107-119.
[12] Esposti, M.D, De-Vries, S, Crimi,M, Ghelli, A, Patarnello, T and Meyer, A. *Mitochondrial cytochrome b: evolution and structure of the protein.* Biochimica et biophysica acta, 1993, 1143 (3) 234-271. http://doi.org/10.1016/0005-2728(93)90197-n
[13] Fenton, M. B. *Observations on the biology of some Rhodesian bats, including a key to the Chiroptera of Rhodesia.* Life Sci. Contr., R. Ont. Mus. 1975, 104, 1-27.
[14] Garcia-Moreno, J, Arctander, P and Fjeldså, J. *Strong diversification at the treeline among Metallura hummingbirds*. The Auk, 1999, 116, 702-711.
[15] Goh, W.L, Siew W.S, Davies S.E.W, Ball. S, Khoo, G, Rahman M.A, Cranbrook E.O. *Genetic Diversity among white nest swiftlet of the genus Aerodramus (Aves:Apodidae: Collocalia) of house- farms in Malaysia*. Raffles Bulletin of Zoology, 2018, 66: 350-360.
[16] Guo, C.T, Takahashi, T, Bukawa, W, Takahashi, N, Yagi, H, Kato, K, Suzuki, Y. *Edible bird's nest extract inhibits influenza virus infection.* Antiviral Research, 2006, 70(3), 140-146.
[17] Hebert, P. D, Stoeckle, M. Y, Zemlak, T. S, Francis, C. M. *Identification of birds through DNA barcodes*. PLoS Biology, 2004, 2(10), e312.
[18] Irwin, D. M, Kocher, T. D, and Wilson, A. C. *Evolution of the cytochrome b gene of mammals*. Journal of Molecular Evolution, 1991, 32(2), 128-144.
[19] Lim C.K and Cranbrook E. *Swiftlet of Borneo: builders of edible nests*. Kota Kinabalu: Natural History Publications (Borneo), 2002.
[20] Lim C.K, and Cranbrook, E. *Swiftlets of Borneo: Builders of Edible Nest*. Natural History Publication (Borneo), 2nd ed Kota Kinabalu. 2014.
[21] Marcone, M.F. *Characterization of the Edible Bird's Nest the 'Caviar of the East'*. Food Research International, 2005, 38(10); 1125-1134.
[22] Marshall, A, and Folley, S. *The Origin of Nest-Cement in Edible Bird Nest Swiftlets (Collocalia spp).* Proceedings of the Zoological Society of London. 2009, *126*(3), 383-390.
[23] Mindell, D. P, and Honeycutt, R. L. *Ribosomal RNA in vertebrates: evolution and phylogenetic applications*. Annual Review of Ecology and Systematics, 1990, 21, 541-566.
[24] Mindell, D. P, Sorenson, M. D, Huddleston, C. J, Miranda Jr, H. C, Knight, A, Sawchuk, S. J, and Yuri, T. *Phylogenetic relationships among and within select avian orders based on mitochondrial DNA*. Avian Molecular Evolution and Systematics, 1997, 213-247.
[25] Nakamura, H. *Forensic species identification based on size variation of mitochondrial DNA hypervariable regions*. International Journal of Legal Medicine, 2009, *123*(2), 177-184.
[26] Norhayati, M, Azman, O, and Wan Nazaimoon, W. *Preliminary study of the nutritional content of Malaysian edible bird's nest*. Malaysian Journal of Nutrition, 2010, *16*(3), 389-396.
[27] Nugroho, E, and Whendrato, I. *The farming of edible-nest swiftlets in Indonesia.* Paper presented at the Technical Workshop on Conservation Priorities and Actions for Edible-nest Swiftlets, Surabaya, Indonesia, 1996.
[28] Parson, W, Pegoraro, K, Niederstätter, H, Föger, M, and Steinlechner, M. *Species identification by means of the cytochrome b gene*. International Journal of Legal Medicine, 2000, 114(1-2), 23-28.
[29] Paton, T. A, and Baker, A. J. *Sequences from 14 mitochondrial genes provide a well-supported phylogeny of the charadriiform birds congruent with the nuclear RAG-1 tree*. Molecular Phylogenetics and Evolution, 2006, 39(3), 657-667.
[30] Rozas J and Rozas R. *DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis*. Bioinformatics Application Note, 1999, 15: 2, 174-175.
[31] Sankaran, R. *The status and conservation of the Edible-nest Swiftlet (Collocalia fuciphaga) in the Andaman and Nicobar Islands*. Biological Conservation, 2001, 97(3), 283-294.
[32] Tamura K, Peterson, D, Peterson N, Stecher G, Nei M and Kumar S. *Molecular Evolutionary Genetics Analysis using maximum Likelihood, evolutionary distance and maximum parsimony methods*. Mol Biol Evol, 2011, 28:2731- 2739.
[33] Thornburn C.C. *The Edible Nest Swiftlet Industry in South East Asia; Capitalism Meets Commensalism*. Human Ecology, 2015, 43: 179-184.

[34] Videler, J, Stamhuis, E, and Povel, G. *Leading-edge vortex lifts swifts*. Science, 2004, 306 (5703), 1960-1962.
[35] Viruhpintu, S, Thirakhupt, K, Pradatsundarasar, A, and Poonswad, P. *Nest-site Characteristics of the Edible-nest Swiftlet Aerodramus fuciphagus (Thunberg, 1812) at Si-Ha Islands, Phattalung Province, Thailand*. Natural History, 2002, 2(2), 31-35.