



Faculty of Resource Science and Technology

**MORPHOLOGY AND GENETIC VARIATIONS OF *Aethalops*
(CHIROPTERA: PTEROPODIDAE) USING MITOCHONDRIAL
AND NUCLEAR GENES**

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**Morphology and Genetic Variations of *Aethalops* (Chiroptera: Pteropodidae) using
Mitochondrial and Nuclear Genes**

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DECLARATION

I hereby declare that no portion of the work referred to this thesis has been submitted in support of an application for another degree or qualification to this or any other university or institute of higher learning.

(Roberta Chaya Tawie anak Tingga)

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ABSTRACT

(*Aethalops* is a mountain endemic fruit bat which consists of two species, namely, *Aethalops aequalis* and *A. alecto*. *A. aequalis* is endemic in Borneo only whereas *A. alecto* can be found on islands in Indonesia and also in Peninsular Malaysia. *A. aequalis* was previously classified as subspecies to *A. alecto* however, most recent study on *Aethalops* suggested *A. aequalis* as a single unit species distinctly different from *A. alecto*. Two approaches used in this study which were molecular and morphometric techniques. The objectives of this study were to clarify the current taxonomic status of *A. aequalis* and its phylogenetic relationship with *A. alecto*.) Populations of *A. aequalis* in Sabah and Sarawak were investigated to detect any genetic variation between the geographic groups and among populations. This study also aimed to determine any morphological variation among the populations and to investigate if morphological variations correlate with molecular genetic distant. The fourth aim of this study was to provide as many data or information that could assist in the conservation management of *Aethalops* in Borneo specifically as this species is endemic mountain bat. Individuals in this study were sampled from seven populations in Sabah and Sarawak. Altogether, there were 70 individuals obtained from fresh samples and voucher specimens. They were categorised into three groups, namely, Southwest Sarawak (Mount [Mt] Penrissen and Mt Pueh), Northeast Sarawak (Mt Murud, Mt Mulu and Bario) and Sabah (Mt Kinabalu and Mt Trus Madi). For morphometric analysis, 65 skulls of adult *A. aequalis* were extracted with 22 cranial and dental characters measured and recorded. Mitochondrial (COI) and nuclear (Rag2) DNA genes were used in this study to infer the genetic variation of *A. aequalis* by applying DNA sequencing technique whereas conserved gene 12S rRNA was used to infer phylogenetic relationships of *Aethalops* from Sundaland. Analyses of results

were interpreted for within and among group variations. Discriminant Function Analysis (DFA) results showed little variation was detected in morphology of Malaysian *A. aequalis*. Characters of distance between lower canine and lower molar (CM1) and distance between lower incisor and lower molar (IM1) were the two strongest characters to discriminate *A. aequalis*. Differences in CM1 (indicating the length of lower jaw) showed a separation between Northeast Sarawak and Sabah (Northern part of Borneo) and Southwest Sarawak groups. Populations from lower elevation mountain at Southwest Sarawak have a longer lower jaw. Longer lower jaw can be related to feeding behavior of the fruit bat. Mt Mulu population showed significant differences in all characters against populations in Mt Penrissen, Mt Kinabalu, Mt Trus Madi and Mt Murud. Morphology differences were suggested to be influenced by the food resources in the environment. Phylogenetic analyses results showed that there was an interspecific genetic divergence in *Aethalops*, separated into two main groups which were *A. alecto* and *A. aequalis*. Using 12S rRNA, Kalimantan population was detected as the possible ancestral population of *A. aequalis* in Borneo. Highly significant genetic differences of *A. aequalis* between populations in Kalimantan and Malaysia Borneo suggested a possibility of two subspecies in Borneo. Both mitochondrial (12S rRNA and COI) and nuclear (Rag2) genes produced unresolved phylogenetic trees at population level. There was little genetic divergence and high gene flow detected among the observed populations in Sabah and Sarawak. However, each of the inferred genes were able to detect that there was a clear pattern in separation time of divergence between Southwest Sarawak and Northeast Sarawak and Sabah groups. It was predicted the population of Malaysian Borneo started from the Southwest Sarawak after diverging from Kalimantan population, then dispersed into Northern part of Borneo during the Pleistocene period. Molecular findings also showed incongruence results with morphological difference between

Mt Mulu and other populations. Overall, *A. aequalis* was a single panmictic population. A single unit of ESUs with two potentially MUs was defined for proper management of conserving this species. Two possible MUs were suggested for populations from Mt Penrissen and Bario that acquire immediate conservation population in future studies with more adequate amount of samples representing each populations. Both of these populations were genetically different from other populations and also were suspected as an ancestral population for *A. aequalis* in Malaysia Borneo based from the preliminary findings. Therefore, it is very important to retain the ancestral haplotype from this population for genetic management purposes in Sarawak. However, more sufficient samples of *A. aequalis* are required to further verify the MUs in Sabah and Sarawak. Current findings on the abundance, morphology and genetic data of *A. aequalis* can be used as baseline information for the conservation and proper management on this species and habitats related to its survival.

Keywords: *A. aequalis*, *A. alecto*, population genetics, morphometric, mitochondrial and nuclear DNA, phylogenetic relationship, genetic variation, ESUs

Analisis Morfologi dan Genetik Variasi Aethalops (Chiroptera: Pteropodidae)

Menggunakan Mitokondria dan Nuklear Gen

ABSTRAK

Aethalops adalah kelawar pemakan buah yang endemik di kawasan pergunungan sahaja. Terdapat dua species *Aethalops* iaitu *Aethalops aequalis* dan *A. lecto*. *A. aequalis* tersebar di Borneo sahaja manakala *A. lecto* didapati tersebar di kepulauan Indonesia dan juga di Semenanjung Malaysia. Sebelum ini, *A. aequalis* telah diklasifikasi sebagai subspecies kepada *A. lecto*. Namun demikian, kajian terkini mencadangkan bahawa *A. aequalis* adalah unit species tunggal yang berbeza dari segi genetiknya dari *A. lecto*. Dalam kajian ini telah mengaplikasikan dua pendekatan iaitu morfologi dan molekular teknik. Salah satu dari objektif kajian ini adalah untuk mengesahkan taksonomi status *A. aequalis* and hubungan filogenetik dengan *A. lecto*. Populasi *A. aequalis* dalam Sabah dan Sarawak juga dikaji untuk mengesan sebarang variasi genetik di antara penghalang geografi dan di kalangan setiap populasi yang dikaji. Selain itu, kajian ini dilihat sama ada wujudnya perbezaan morfologi dalam *A. aequalis* dan kemudian dibandingkan dengan hasil molekular. Salah satu kepentingan kajian adalah untuk pengurusan konservasi spesis ini. Individu dalam kajian ini disampel dari tujuh populasi di Sabah dan Sarawak. Populasi tersebut telah dikategorikan dalam tiga kumpulan iaitu Barat Daya Sarawak (Gunung Penrissen dan Gunung Pueh), Timur Laut Sarawak (Gunung Murud, Gunung Mulu dan Bario) dan Sabah (Gunung Kinabalu dan Gunung Trus Madi). Keseluruhan terdapat 70 individu *A. aequalis* berjaya diperoleh dari sample baru dan spesimen muzium. Sebanyak 65 tengkorak *A. aequalis* telah diekstrak untuk analisa morfologi dan 22 ciri tengkorak dan gigi diukur dan direkodkan.

Mitokondrial (12S rRNA dan COI) dan nuklear (Rag2) gen untuk menerangkan genetik variasi dengan teknik DNA sequencing. Analisa fungsi diskriminasi ('Discriminant Function Analysis') menunjukkan perbezaan variasi morfologi yang sangat kecil di kalangan A. aequalis Borneo Malaysia. Ciri jarak antara gigi taring bawah dengan molar bawah (CM1) dan jarak antara gigi kacip bawah dengan molar bawah (IM1) merupakan ciri yang terbaik untuk tengkorak dan gigi. Terdapat perbezaan dalam kepanjangan ciri CM1 (merujuk kepada kepanjangan rahang gigi bawah) telah dikenalpasti di antara kumpulan Timur Laut Sarawak dengan kumpulan Barat Daya Sarawak dan Sabah (Utara Borneo). Analisa menunjukkan bahagian Timur Laut Sarawak dengan ketinggian gunung yang lebih rendah mempunyai rahang gigi yang lebih panjang. Kepanjangan dalam rahang gigi mempunyai hubungkait dengan kelakuan pemakanan. Gunung Mulu menunjukkan perbezaan yang signifikan dengan populasi Gunung Kinabalu, Gunung Trus Madi, Gunung Murud dan Gunung Penrissen dalam semua ciri tengkorak dan gigi yang dikaji. Perbezaan morfologi dicadangkan dipengaruhi oleh sumber makanan yang ada di kawasan persekitaran. Filogeni menunjukkan bahawa terdapat perpisahan genetik dalam Aethalops dengan terdapatnya dua kumpulan utama iaitu A. aequalis dan A. alesto. Populasi Kalimantan telah dikenalpasti sebagai populasi asal A. aequalis. Perbezaan yang signifikasi dari segi genetik variasi A. aequalis antara populasi di Kalimantan dan Malaysia Borneo mencadangkan bahawa kemungkinan kehadiran dua subspecies A. aequalis di Borneo. Kedua – dua mitokondrial (COI dan 12S rRNA) dan nuklear (Rag2) gen menghasilkan pokok filogenetik yang tidak sempurna penyelesaiannya pada peringkat populasi. Hanya sedikit genetik variasi dengan aliran gen yang sangat tinggi dikesan dalam semua populasi yang telah dikaji. Walau bagaimanapun, setiap gen yang digunakan berupaya menghasilkan corak perpisahan berdasarkan masa evolusi yang jelas di antara kumpulan Barat Daya Sarawak dan Sabah

dengan kumpulan Timur Laut Sarawak. Selepas berpisah dari populasi Kalimantan, populasi *A. aequalis* Borneo Malaysia dijangkakan bahawa berasal dari Timur Laut Sarawak, dan tersebar ke bahagian Utara Borneo semasa peristiwa ‘Pleistocene’. Kajian ini menunjukkan hasil analisa morfologi tidak memberi corak pembahagian kumpulan yang sama dengan hasil analisa molekular. Secara keseluruhan, *A. aequalis* diklasifikasi sebagai satu unit ‘panmitic’. Species *A. aequalis* dikelaskan sebagai satu unit ‘Evolutionary Significant Units (ESUs)’ dengan dua potensi unit ‘Management Unit (MUs)’ telah dikenalpasti sebagai langkah pengurusan teratur untuk pemuliharaan species *A. aequalis*. Gunung Penrissen dan Bario telah berpotensi dikenali sebagai ke ‘MU’ agar pemuliharaan species ini dijalankan secara berasingan dengan sampel individu lebih banyak untuk mewakili populasi tersebut. Kedua – dua populasi tersebut mempunyai perbezaan genetik variasi yang jauh berbeza berbanding dengan populasi – populasi yang lain dan diramal sebagai susur-galur kepada semua populasi di Sabah dan Sarawak, berdasarkan penemuan awal dalam kajian ini. Jadi, ia amatlah penting untuk mengekalkan ‘haplotype’ keturunan dari populasi ini. Lebih banyak sampel diperlukan dalam kajian yang akan datang untuk pengenalpastian yang selanjutnya. Segala maklumat mengenai kelimpahan, morfologi dan genetik *A. aequalis* akan digunakan sebagai data asas dalam pemeliharaan serta panduan untuk pengurusan yang sistematis bagi spesis *A. aequalis* dengan habitat yang berkenaan untuk kemandirian kelawar ini.

Kata Kunci: *A. aequalis*, *A. alecto*, genetik populasi, morfometrik, mitokondrial dan nuklear DNA, hubungan filogenetik, genetik variasi, ‘ESUs’

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