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Phylogeny and Phylogeography of *Myotis muricola* (Gray, 1846) (Chiroptera: Vespertilionidae) from the West and East of Wallace's Line Inferred from Partial MtDNA Cytochrome *b* Gene

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ABSTRACT

Myotis muricola is a widespread species covering the Malay Archipelago through the West and East of Wallace's Line. The genetic analysis, based on partial cytochrome *b* gene, shows the high genetic variation within *M. muricola*. The phylogenetic analysis has indicated that *M. muricola* in the Malay Archipelago are monophyletic. Members of *M. muricola* Eastern are grouped together independently of *M. muricola* Western and both groups are distantly related. On the other hand, *M. muricola* Western and *M. muricola* Eastern are distinct species and sister taxa to *M. mystacinus*. Based on the high genetic distance (26.8% to 38.5%) and the Genetic Species Concept (Baker & Bradley, 2006), it can be concluded that *M. muricola* Western and *M. muricola* Eastern should be considered as two distinct species. Furthermore, two subgroups within *M. muricola* Western, namely Sumatra-Asian and Bornean subgroups, are recognised as distinct subspecies (with genetic distance of 5.1% to 10.8%). The evidence from the molecular data indicated *M. muricola* Eastern as the ancestor of *M. muricola* species complex in the Malay Archipelago, which had earlier diverged into the western region during the Pliocene. Meanwhile, the geographical conditions during the Pleistocene had given more chances for fauna to diversify. It was predicted that *M. muricola* diverged in the western part of the Malay Archipelago during the Pleistocene when the sea level dropped and produced some landbridges among the islands in Sundaland. The hypothetical dispersal routes of *M. muricola* are related to the ancient Sunda River systems that produced gallery forest corridors for migration and which served as Pleistocene refuges during the migration.

Keywords: Chiroptera, *Myotis muricola*, Phylogeny, Phylogeography, cytochrome *b*, Malay Archipelago

INTRODUCTION

The Malay Archipelago region consists of many islands which have the richest biodiversity in the world (Myers *et al.,* 2000). It has many features that tremendously contribute to evolutionary biology studies, particularly the linear island geography which provides natural boundaries to population ranges and has environmental gradients (Maharadatunkamsi *et al*., 2000). Furthermore, the region is one with the gradual physical transitions, typified by a decline in

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rainfall from the west to east (Oldeman *et al.,* 1980), and it has a lot of barriers such as sea. In addition, this archipelago was divided by Wallace's Line into two regions, namely the West and East of Wallace's Line. In the northern part, this line lies between Borneo and Sulawesi Islands. Meanwhile, the Lombok Strait represents the Wallace's Line that divides Bali Island as a part of the western region and Lombok Island as a part of the eastern region in the southern part. The archipelago has an unstable and complicated geological history, experiencing marked changes in the recent past, and a cline in many environmental variables that exists from the west to east, along the archipelago which may influence the distribution, habitat and genetics of its faunal elements (Mayr, 1976). These conditions are associated with rapid speciation processes (Whitmore, 1987) and promote high genetic variations among the species in this region. Some previous studies on bats in this region have revealed that there is a strong gradual cline in genetic variations, such as *Cynopterus nusatenggara* (Schmitt *et al.*, 1995), *Eonycteris spelaea* (Hisheh *et al.*, 1998), *Rhinolophus affinis* (Maharadatunkamsi *et al.*, 2000), two Vespertilionid bats (Hisheh *et al.*, 2004), Macroglossinae species (Jayaraj, 2008) and Malaysian *Kerivoula* (Faisal, 2008; Hasan, 2008).

Myotis muricola is a widespread species that is known from Southeast Asia, from East India to South China, and Indonesia, as well as from Sumatra through the Moluccas and the Philippines (Corbet & Hill, 1992; Suyanto, 2001; Simmons, 2005; Francis, 2008). Taxonomically, based on morphological similarities, *M. muricola* was first described as *Vespertilio muricola* by Hodgson in 1841 (*nomen nudum*), and then by Gray in 1846, with the same name. Meanwhile, Chasen (1940) called most Sundanesian specimens as *M. m. muricola* but employed *niasensis* as a valid subspecies of *M. muricola* for those collected from the Nias Island. Tate (1941), however, suggested that all should be referred to as *M. mystacinus* for the specimens from Sumatra, Java, Borneo, and allocated *niasensis* as a subspecies known as *M. mystacinus niasensis*. Laurie and Hill (1954) followed the lead by Tate (1941) in employing *M. mystacinus* but used *muricola* as the sub-specific name for the specimens from the Lesser Sunda Islands and by implication from much of Sundanesia. Corbet (1978) suggested that *M. mystacinus* and *M. muricola* should be considered specifically distinct. According to van Strien (1986), there are three sub-species of *M. muricola* in Indonesia, and these are *M. m. muricola* (Sumatra, Sipora, Java, Bali, Borneo, Sumba, Sumbawa and Flores), *M. m. browni* (Central Sulawesi) and *M. m. niasensis* (Nias Island). Recently, the classification by Simmons (2005) outlines that *M. muricola* as a distinct species from *M. mystacinus*. It is important to note that *M. mystacinus* does not extent eastwards into Asia beyond Kazakhtan, Syria and Israel. This taxon has been replaced by *M. nipalensis* which is distributed from Iran, Turkey, and Uzbekistan to Nepal, Mongolia, Tibet, NW China and Siberia. Furthermore, the specimen from Vietnam which had originally identified as *M. mystacinus* might represent as *M. muricola* (*see* Bates *et al.*, 1999). On the other hand, Bates *et al.* (2005) recorded *M. mystacinus* from Myanmar as the first authenticated one for Southeast Asia. Therefore, comprehensive studies on *Myotis* are very much needed.

The widely distributed species in the Malay Archipelago shows a genetic structuring that is associated with geography (Hisheh *et al.*, 2004). Previous systematic studies on *M. muricola* have been based on morphological characters, but the systematic status of this species is still unstable and represented as species complex (Francis, 2008). Meanwhile, Faisal (2008) has suggested that there are more than one species under this name, based on a genetic study. Unfortunately, there is no detailed genetic study on *M. muricola* in the Malay Archipelago. Only one genetic study of *M. muricola* has been recorded for Kalimantan and the Lesser Sunda Islands and this was done by Hisheh *et al.* (2004) using allozymes that showed strong associations between genetic and geographical parameters and overall greater genetic variability as measured by mean island heterozygosities.

Over the last two decades, a broad-based DNA sequence data set has become available to examine the biodiversity and speciation in mammals (Baker & Bradley, 2006). Systematicists began to use DNA sequences to study the phylogenetic relationships among taxa, whereas population biologists began to evaluate phylogeographic patterns in DNA sequence variation among individuals within a single species (Avise, 2000). Mitochondrial DNA (mtDNA) offers a particularly valuable source of markers for the study of closely related taxa (Funk & Omland, 2003) because mtDNA evolves rapidly in population of higher animals and it is usually transmitted maternally without intermolecular recombination (Avise, 1998). The cytochrome *b* (cyt *b*) gene has been used in numerous studies of phylogenetic relationships within mammals and its sequence variability makes it the most useful for the comparison of species. Although Galtier *et al.* (2009) have suggested that mtDNA is perhaps intrinsically the worst population genetic and phylogenetic molecular marker, the authors agree that this particular issue is still under constant debate. Therefore, this paper follows some previous studies by Ruedi and Mayer (2001), Piagio *et al*. (2002), Kawai *et al*. (3003), Baker and Bradley (2006), Stadelmann *et al.* (2007), Garcia-Mudara *et al.* (2009), in which cytochrome *b* is stated as a useful marker for molecular study of mammals, especially in bats.

Thus, the aim of this study was to examine the phylogenetic relationships within *M. muricola*, between *M. muricola* and *M. mystacinus* and resolve their taxonomic status using the partial mitochondrial DNA cytochrome *b* gene sequences. Furthermore, this study was

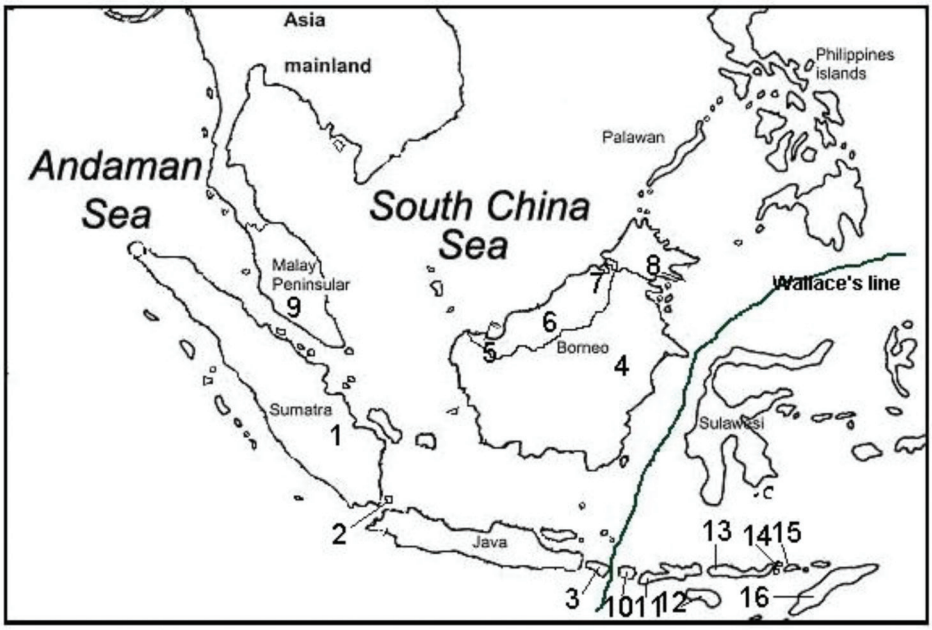

Fig. 1: The localities of the specimens of M. muricola *that were examined. The west of Wallace's Line region is represented by (1) Jambi (2) Krakatau Island (3) Bali Island (4) East Kalimantan (5) Southern Sarawak (6) Central Sarawak (7) Northern Sarawak (8) Tawau, Sabah (9) Fraser Hill, Pahang. The sites from the east of Wallace's Line include: (10) Lombok Island (11) Sumbawa Island (12) Sumba Island (13) Flores Island (14) Lembata Island (15) Pantar Island (16) Timor Island*

also aimed to reconstruct the phylogeography of *M. muricola* species complex in the Malay Archipelago, based on their genetic analyses.

MATERIALS AND METHODS

Study Area

The study area covered the localities from many islands that represented the two different regions, namely, the West and East of Wallace's Line. This study area was divided politically into two countries, with a bigger part of these sampling sites being from Indonesia and the remaining sites were chosen from Malaysia. These sampling sites were chosen based on the distributions of *M. muricola* in the Malay Archipelago. Representative samples from throughout the geographic range, including small islands, were needed to examine the genetic variations of the populations as well as the biogeography of *M. muricola*. Generally, this study was based on the available museum collections. However, some fieldworks were also conducted to cover the geographic range of this particular species. The sampling sites of this study are shown in *Fig. 1*.

DNA Samples and Other Sequence Resources

Genetic materials (liver and tissue) of *M. muricola* were taken from the specimen collections in Museum Zoologicum Bogoriense (MZB), Museum Zoological Universiti Malaysia Sarawak (MZU) and additional fieldwork to acquire the fresh samples in Sarawak and Peninsular Malaysia (Table 1). All of the specimens from the museum and fieldwork were re-identified. The identification of the specimens were according to the identification key by Corbet and Hill (1992), Suyanto (2001), Yasuma *et al.* (2003) and Francis (2008). In particular, *M. muricola* was classified into the sub-genus *Selysius* which differed from the other sub-genus by having small feet (including claw < ½ of the tibia length) with the wing membrane that usually is attached at the base of the toes (Corbet & Hill, 1992; Yasuma *et al.*, 2003). Furthermore, *M. muricola* differs from the other species within the subgenus *Selysius* by having upper part brown to grey with dark bases, under parts with dark bases, and light brown tips. Ears are moderately long, tragus slender, bent forwards and bluntly pointed. The feet are small with wing membranes attached at the base of the toes (Francis, 2008). The length of the forearm ranges from 30-37 mm with three pairs of premolar. Braincase is flatter, and it is not rising abruptly from the rostrum. The condylobasal length is 12.7-13.1 mm and condylocanine length is 11.2-12.6 mm. The second upper premolar is small, rarely very small, and is usually in row or slightly intruded. Meanwhile, the second lower premolar is usually in row, and it is sometimes slightly intruded (Corbet & Hill, 1992; Suyanto, 2001; Yasuma *et al.,* 2003). Unfortunately, a lot of specimens from the collections of MZB were unsuccessfully extracted for DNA because they were preserved in formalin as the preservative chemical when these samples were collected during the fieldwork, although they were then transferred into ethanol in the museum. The DNA was already degraded by this chemical. If the DNA was still in the tissue and it was more a matter of getting it out than it being chopped up (White & Densmore, 1992). However, only eight samples of *M. muricola* which had been contaminated by formalin were successfully extracted. Some sequences from the previous studies were included in this study, and these included several sequences of *M. mystacinus* to construct the relationships among the *M. muricola* populations. All of these additional sequences were taken from GenBank (Table 2).

DNA Extraction

Total DNA was extracted using the CTAB (cetyl-tri-methyl ammonium bromide) protocol (Ducroz *et al.*, 1998; Lecompte *et al.*, 2005). 700 µl 2X CTAB buffer and 8 mg of proteinase K were added into the 1 cubic millimetre minced tissue samples to denature the proteins to eliminate the proteins which could affect the DNA product (Di Mito & Betschart, 1998). Then, 700 µl of chloroform-isoamyl alcohol was added to inhibit

TABLE 1
List of the specimens, museum reference, location, habitat, abbreviation (Abbr.) and GenBank accesion numbers

Species	Museum reference	Location		Habitat	Abbr.	GenBank Accesion No.
		Geographical region	Locality of specimen			
M. muricola	MZB 28109	Southern Sumatra	Jambi	secondary forest	Mm Jambi1 SM	GU358630
M. muricola	MZB 28110	Southern Sumatra	Jambi	secondary forest	Mm Jambi2 SM	GU358631
M. muricola	MZB 28111	Southern Sumatra	Jambi	secondary forest	Mm Jambi3 SM	GU358632
M. muricola	MZB 16999	Krakatau	Krakatau Island	na.	Mm Kr	GU258636
M. muricola	MZB 23629	Eastern Kalimantan	Long Bawan, Kayan Mentarang	agricultural plantation	Mm EK	GU358635
M. muricola	TK 153691*	Peninsular Malaysia	Fraser Hill, Pahang, Malaysia	mixed dipterocarp forest	Mm PahangPM	GU358633
M. muricola	MZU/M/00321	Southern Sabah	Tawau Hill	lowland dipterocarp forest	Mm Sbh	GU358634
M. muricola	MZU/M/00302	Southern Sarawak	Kubah National Park	mixed dipterocarp forest	Mm KubahSWK	GU358641
M. muricola	MZU/M/00303	Southern Sarawak	Kubah National Park	mixed dipterocarp forest	Mm Kubah2SWK	GU358642
M. muricola	MZU/M/00767	Southern Sarawak	Jambusan cave, Bau limestone	secondary forest	Mm BauSWK	GU358639
M. muricola	MZU/M/00504	Southern Sarawak	Mount. Pueh, Sematan	mixed dipterocarp forest	Mm PuehSWK	GU358640
M. muricola	TK 153582*	Central Sarawak	Bukit Lima, Sibu	lowland dipterocarp forest	Mm SibuSWK	GU358643
M. muricola	TK 153649*	Central Sarawak	Menyarin River, Lanjak Entimau	mixed dipterocarp forest	Mm LanjakSWK	GU358644
M. muricola	MZU/M/00559	Central Sarawak	Similajau, Bintulu	lowland dipterocarp forest	Mm SmjSWK	GU358638
M. muricola	MZU/M/01576	Northern Sarawak	Niah National Park	lowland dipterocarp forest	Mm NiahSWK	GU358637
M. muricola	MZB 20009	Western Lesser Sunda	Bali Island	na.	Mm Bali	GU358646
M. muricola	MZB 19931	Western Lesser Sunda	Lombok Island	deciduous forest	Mm Lombok	GU358648
M. muricola	MZB 19979	Central Lesser Sunda	Sumbawa Island	deciduous forest	Mm Sumbawa	GU358651
M. muricola	MZB 19996	Central Lesser Sunda	Sumba Island	deciduous forest	Mm Sumba	GU358645
M. muricola	MZB 20034	Eastern Lesser Sunda	Flores Island	deciduous forest	Mm Flores	GU358647
M. muricola	MZB 20055	Eastern Lesser Sunda	Lembata Island	deciduous forest	Mm Lembata	GU358649
M. muricola	MZB 20049	Eastern Lesser Sunda	Pisang, Pantar Island	deciduous forest	Mm Pantar	GU358650
M. horsfieldii	MZU/M/01599	Northern Sarawak	Niah National Park	lowland dipterocarp forest	*M. horsfieldii*	GU358652

Note: (na.) data were not available, *field number (has not been registered at museum reference)

TABLE 2
Additional mtDNA cytochrome *b* sequences from the GenBank

Species	Locality	Abbr.	GenBank	
			Authors	Accession No.
M. muricola A	Asia mainland	Mm A	Tsytsulina *et al.* (2004)	AY665143
M. muricola B	Asia mainland	Mm B	Tsytsulina *et al.* (2004)	AY665144
M. muricola C	Bam Keng Bit, Laos	Mm C	Stadelmann *et al.* (2004)	AJ841957
M. muricola browni	Mindanau, Philippines	Mm browni	Ruedi and Mayer (2001)	AF376589
M. mystacinus A	Europe	Mmt A	Ibanez *et al.* (2006)	DQ120879
M. mystacinus B	Europe	Mmt B	Ibanez *et al.* (2006)	DQ120880
M. mystacinus C	Europe	Mmt C	Ibanez *et al.* (2006)	DQ120881
M. mystacinus D	Morocco	Mmt D	Garcia-Mudarra *et al.* (2009)	EU360642
M. mystacinus E	Morocco	Mmt E	Garcia-Mudarra *et al.* (2009)	EU360643

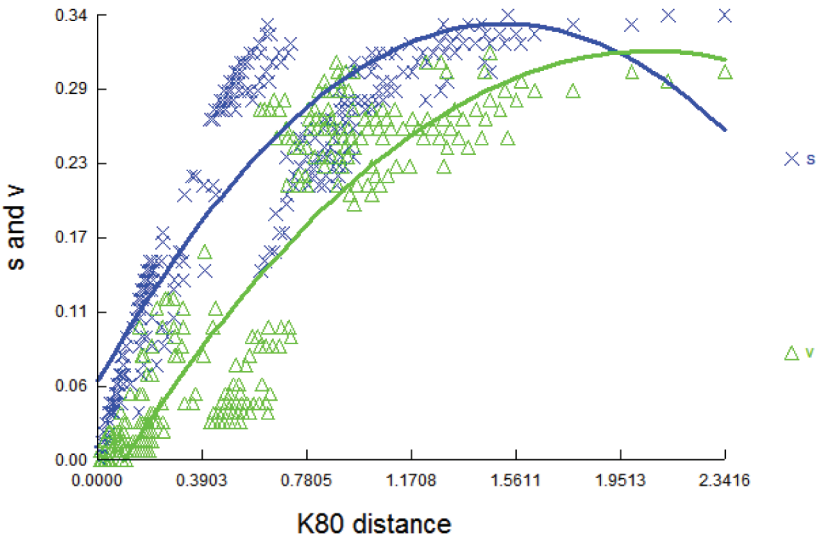

Fig. 2: Saturation plots (the total number of substitution both in the transition and tranversion versus the total amount of uncorrected sequence divergence) calculated using the Kimura two-parameter model (Kimura, 1980)

the lysis process, which was done by proteinase K, while incubating in a water bath at 60°C for 1-3 hours. After centrifugation at 13000 rpm for 10 minutes, 500-550 µl of the upper aqueous layer containing DNA was transferred into a 1.5 ml fresh microcentrifuge tube. Absolute ethanol of the same amount was also added. The absolute ethanol was used to precipitate the DNA product and avoid interference by other products (Gari *et al.,* 2006). After the second centrifugation, the supernatant was removed into a new tube with 600 µl cold 70% ethanol and 25 µl 3M NaCl. Following the third centrifugation, the excess ethanol was discarded and the pellet was air-dried. Finally, the DNA was resuspended in 30 µl of double distilled water (ddH_2O). The extracted DNA samples were kept at -80 ºC in a freezer for later use.

Amplification and Sequencing

25 µl of reaction volume was used for the PCR amplification. This volume included 1 µl of DNA extract, 1.2 µl of each primer pair (10 mM Glud-GL and 10 mM CB2H), 1.5 µl of 25 mM $MgCl_2$, 0.5 µl of 10 mM dNTPs, and 0.5 µl of *Taq* DNA polymerase with appropriate buffer and ddH_2O to volume. Meanwhile, the thermal profiles of amplifications started with three minutes of denaturation at 93°C, followed by 30 cycles at 93°C (1 min), 47.2°C (1 min 15 s) and 72°C (2 min), with a final extension at 72°C (5 min). To amplify the partial cytochrome *b* gene, the primers used were GludG-L, (5'-TGACCTGAARAACCAYCGTTG-3') and CB2H (5'-CCCTCAGAATGATATTTG TCCTCA-3') (Palumbi *et al.,* 1991). The purification of the PCR product was carried out using the Promega Purification Kit, following the protocol provided by the manufacturer (Wizard® Genomic DNA Purification Kit-Instruction for use of product). The purified PCR products were sequenced by a private laboratory (1st Base, Selangor, Malaysia) using the ABI 3730 Genetic Analyzer.

Phylogenetic Analysis

CHROMAS version 1.45 (MacCarthy, 1996) was used to display the fluorescence nucleotide bases of the DNA sequence for the analysis. Multiple sequence alignments were done by using CLUSTAL X version 1.81 programme (Thompson *et al.*, 1997) and subsequently aligned by eye. Molecular Evolutionary Genetics Analysis (MEGA version 3) (Kumar *et al.,* 2004) was used to perform the analysis of base frequencies and distance matrix. Genetic divergence (Saitou & Nei, 1987) analysis was carried out using Kimura two-parameter model (Kimura, 1980). Meanwhile, the sequence saturation analysis was done using DAMBE version 5.0.7 (Xia & Xie, 2001) by plotting the total number of substitutions against the total amount of uncorrected sequence divergence which was calculated using Kimura's two-parameter model (Kimura, 1980).

Phylogenetic relationship was constructed using four methods; namely, neighbour-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference. The NJ method was implemented using the Kimura two-parameter as the model of evolution (Kimura, 1980), whereas, the weighted MP analysis was done using full heuristic searches, tree-bisection-recognition (TBR) branch-swapping and random stepwise addition. ML inference applied the best-fit maximum likelihood model of sequence evolution using the Akaike information criterion (AIC) in Modeltest 3.7 (Posada & Crandall, 1998). NJ, MP and ML implemented in the Phylogenetic Analysis Using Parsimony (PAUP*) Version 4.0b4 (Swofford, 1998) and the pylogenetic confidence was estimated by bootstrapping (Felsenstien, 1985), with 1000 replicate data sets for the NJ and MP methods, except for the ML method with 100 replicate data sets. Furthermore, the Bayesian inference implemented in MRBAYES 3.0b4 (Ronquist & Huelsenbeck, 2003) was used to estimate a phylogeny by applying different models of molecular evolution for each partition of the molecular data, based on the substitution

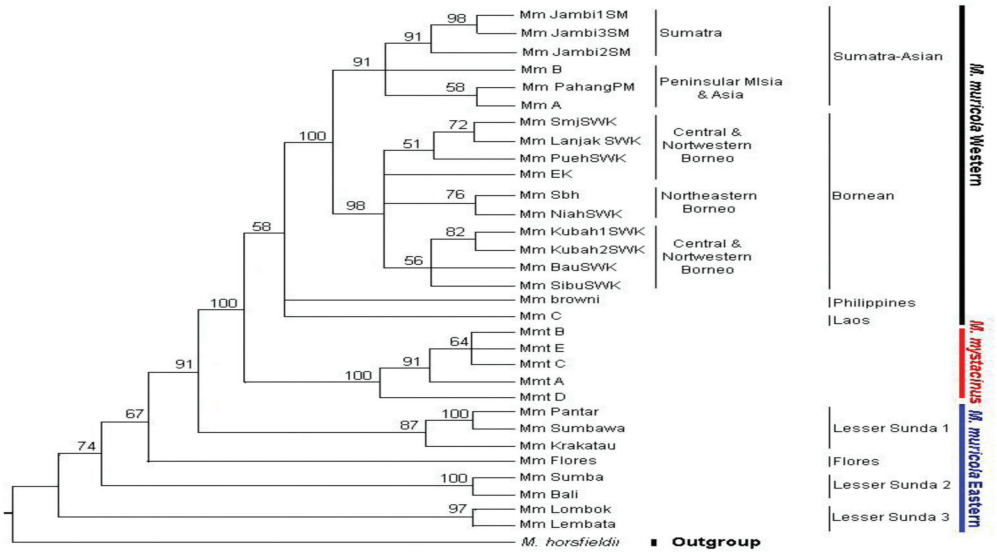

Fig. 3: Neighbour-joining (NJ) tree showing the relationships between M. muricola *vs.* M. mystacinus *and between the groups and sub-groups within the* M. muricola *populations based on the partial cytochrome b mtDNA and calculated using the Kimura two-parameter model of evolution (Kimura, 1980). The values on the branches represent NJ bootstrap values, based on 1000 replicates. Only the bootstrap values >50% are shown. Meanwhile, species abbreviations are given in Tables 1 and 2*

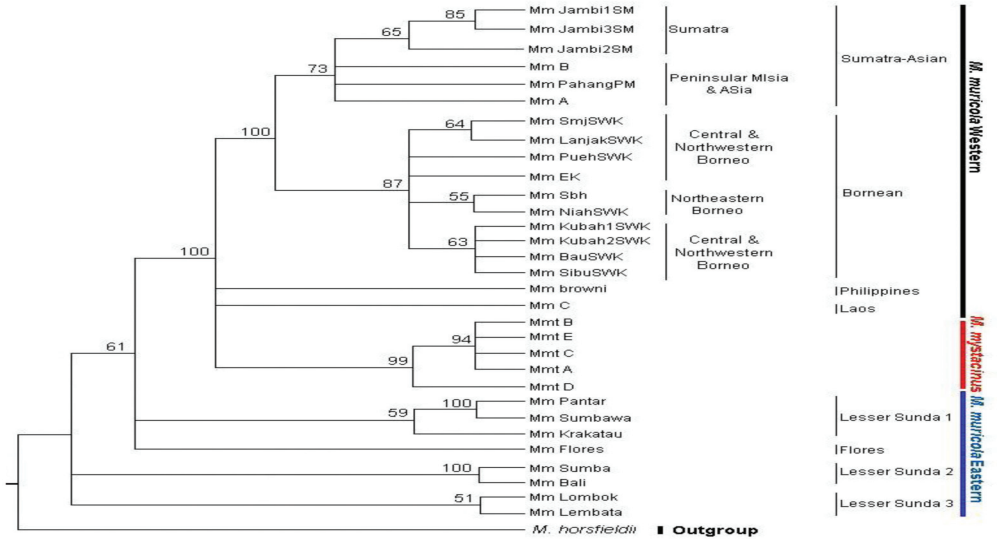

Fig. 4: The weighted and rooted Maximum parsimony (MP) tree showing the relationships between M. muricola *vs.* M. mystacinus *and between the groups and sub-groups within* M. muricola *populations based on the partial cytochrome b mtDNA. The values on the branches represent MP bootstrap values, based on 1000 replicates. Only bootstrap values >50% are shown. Species abbreviations are given in Tables 1 and 2*

model and phylogenetic parameters identified as optimal by AIC criterion in Modeltest 3.7 (Posada & Crandall, 1998). The time of divergence of bats in this study was estimated following (Irwin *et al.*, 1991) the evolutionary rate of cytochrome *b* gene as 0.2% substitution per million years (Myr) and was calculated based on the Kimura two-parameter distance matrix implemented in MEGA version 3 (see above).

RESULTS

Sequences Analysis

A total of 32 partial sequences of 412 bp each of cytochrome *b* were obtained and used in the phylogenetic analysis of this study. These sequences consisted of 26 sequences of *M. muricola*, five sequences of *M. mystacinus* and one sequence of *M. horsfieldii* as an out-group. The average of the nucleotide composition was T (31.0%), A (28.5%), C (24.4%) and G (16.1%). According to Briolay *et al*. (1998) and Bastian *et al*. (2001), the composition of nucleotide showing the anti-G bias is one of the characteristics of mitochondrial DNA. From 412 bp sequences, 200 (48.5%) were variable sites and 152 (76%) were parsimoniously informative sites. Among 31 sequences of *M. muricola* and *M. mystacinus*, 29 haplotypes were identified and 27 were regarded as unique, while two others shared haplotypes within each group; namely, haplotype 1 (Mm Jambi1SM and Mm Jambi3SM) and haplotype 18 (Mmt B and Mmt E). Nonetheless, no haplotype was shared among the groups.

Saturation plots (*Fig. 2*) revealed a high degree of saturation in the third position codon. This was indicated by the fitted curves for transversion having reached the same level as transition. Therefore, the maximum parsimony trees were generated by characters-weighting strategy (with all the characters equally weighted). Furthermore, the maximum likelihood and Bayesian inferences applied the best-fit model of sequence evolution from within the range of models evaluated by script MrAIC (Nylander, 2004) using the Akaike information criterion (AIC). The models that partitioned the rates of change by codon positions were best to account for the sequence characteristics that obscured phylogenetic signal, such as the saturation in substitutions and significant differences in base composition. These models also ameliorated (but did not entirely avoid) the impact of taxon sampling on phylogeny reconstruction (Davalos & Perkins, 2008). Based on the AIC, by using Modeltest 3.7 (Posada & Crandall, 1998), the best-fit model K81uf+G (Kimura, 1981) was applied for the maximum likelihood and Bayesian inferences in this study.

The average pairwise distance (Table 3) was calculated using the Kimura two-parameter model (Kimura, 1980). The pairwise

TABLE 3
Average pairwise genetic distance matrix between the groups and sub-groups calculated using the Kimura two-parameter model (Kimura, 1980)

	M. muricola Western	*M. muricola* Eastern	*M. mystacinus*	Sumatra-Asian
Major group				
M. muricola Western	-			
M. muricola Eastern	0.315	-		
M. mystacinus	0.171	0.26	-	
Sub-group				
Sumatra-Asian	-	0.311	0.168	-
Bornean	-	0.318	0.175	0.072

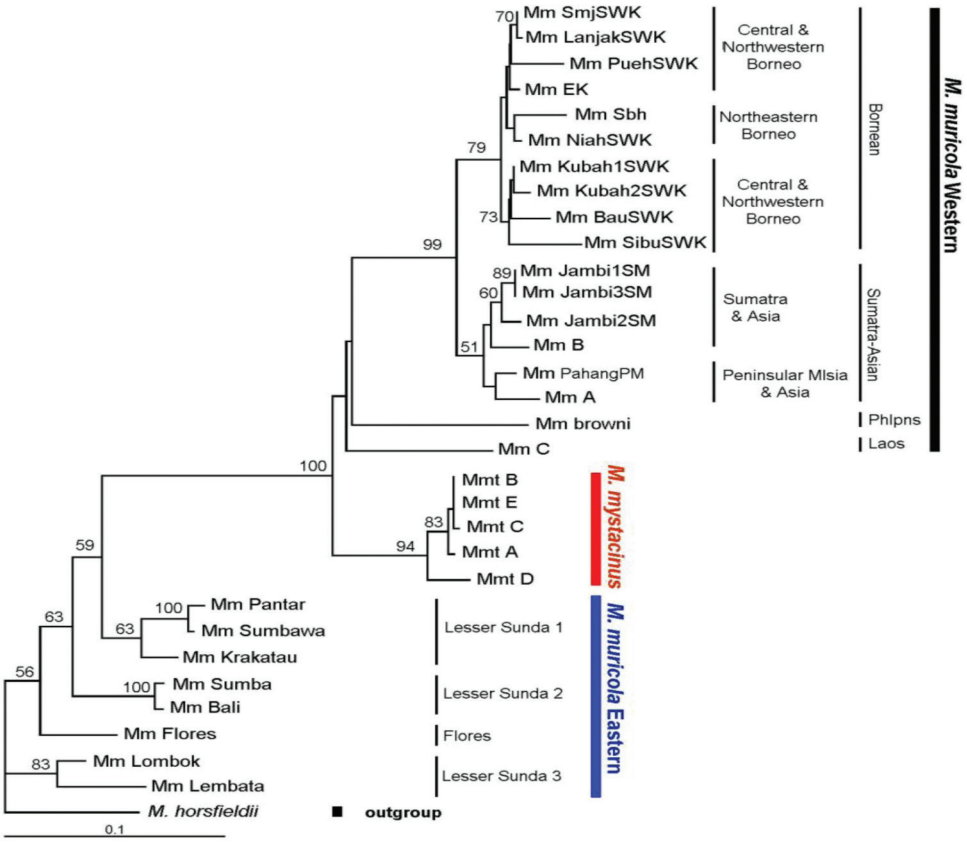

Fig.5: The maximum likelihood (ML) tree showing the relationships between M. muricola *vs.* M. mystacinus *and between the groups and the sub-groups within* M. muricola *populations based on the partial cytochrome* b *mtDNA. The maximum likelihood inference applied the best-fit model of sequence evolution, K81uf+G from the Akaike information criterion (AIC) using Modeltest 3.7 (Posada & Crandall, 1998). The values on the branches represent ML bootstrap values, based on 100 replicates. Only the bootstrap values >50% are shown. Species abbreviations are given in Tables 1 and 2*

comparisons among all the sequences (including the out-group) ranged from 0% to 39.9%. In this study, no sequence from Java Island was available. Generally, all the sequences of *M. muricola* formed two major groups, with the Western group named as *M. muricola* Western consisting of the sequences from Sumatra, Peninsular Malaysia, the Asian mainland (AY665143, AY665144 and AJ841957) and the Philippines with an average genetic distance of 8% (ranging from 0% to 21% in differences within this group). The second group, referred to as *M. muricola* Eastern, was formed by the sequences from Krakatau, Bali, Lombok, Sumba, Sumbawa, Flores, Lembata and Pantar, with an average genetic distance of 9.5% (ranging from 1% to 16.4%). Within *M. mystacinus*, on the other hand, the average genetic distance was 1.5% (ranging from 0% to 3.5%).

The average genetic distance between *M. mystacinus* and *M. muricola* was 17.1%, (ranging from 13.9% to 19.7%, with *M. muricola*

Western) and 26% (ranging from 23.2% to 30.9%, with *M. muricola* Eastern). Within *M. muricola*, among the Western and Eastern, showed a high average genetic distance of 31.5% ranging from 26.8% to 38.5%. Furthermore, within *M. muricola* Western, the specimens from Sumatra, Peninsular Malaysia and mainland Asia formed one sub-group (namely Sumatra-Asian subgroup), whereas the specimens from Borneo became one group (namely Bornean subgroup). The average genetic distance between these two sub-groups is 7.2%, which ranged from 5.1% to 10.8%.

Phylogenetic Analysis

The phylogenetic trees were constructed using NJ, MP, ML and Bayesian inferences, as illustrated in *Fig. 3*, *4*, *5* and *6*, respectively. The maximum parsimony basal clade of the phylogeny was *M. muricola* Eastern, which consisted of *M. muricola* from Lesser Sunda (Bali, Lombok, Sumba, Sumbawa, Pantar, Lembata and Flores) and Krakatau. This particular group diverged from the other two groups around 5.2 Mya. The separation of this group was supported by low bootstrap value in MP, ML and Bayesian (61% for MP, 59% for ML and 57% for Bayesian), but a higher bootstrap value in NJ (91%).

M. muricola Western, which consisted of the sequences from Sumatra, Peninsular Malaysia, Asian mainland, Borneo and Philippines, is a sister species to the *M. mystacinus* group and it diverged around 3.4 Mya. The relationship between these groups is supported with high bootstrap values (100% in all the analyses).

M. muricola Western consisting of the two big sub-groups was fully resolved by using the NJ, MP, ML and Bayesian methods. The branching topology between the two sub-groups was supported by high bootstrap values (100% for NJ, MP, Bayesian and 99% for ML). The Sumatra-Asian sub-group was formed by the sequences from Sumatra, Peninsular Malaysia and two sequences from the Asian mainland (AY661543 and AY661544). On the other hand, the Bornean sub-group consisted of the sequences from the Borneo Island (Sarawak, Sabah and Kalimantan). It is important to note that the separation of these sub-groups was estimated to be around 1.44 Mya.

DISCUSSION

Three major groups were resolved in all of the phylogenetic tree analyses, and based on NJ, MP, ML and Bayesian, the *M. muricola* species formed in the Malay Archipelago are reciprocally monophyletic. At the basal of the phylogenies is *M. muricola* Eastern, which consists of *M. muricola* from Lesser Sunda, which is thus hypothesised as the ancestor of *M. muricola* in the Malay Archipelago. Members of *M. muricola* Eastern are clustered independently of *M. muricola* Western and both the groups are distantly related. On the other hand, all the phylogenies also showed the sister relationship of *M. muricola* Western and *M. mystacinus.*

Phylogenetic Relationship of M. muricola *and the Taxonomic Implications*

Based on the phylogenetic trees, the relationship between *M. muricola* and *M. mystacinus* was fully resolved and supported with high bootstrap value (100% for all the methods) and *M. muricola* Western is a sister species of *M. mystacinus*. Furthermore, *M. mystacinus* also shows a high genetic distance from *M. muricola*. The average genetic distance between *M. mystacinus* and *M. muricola* was found to be 17% (with *M. muricola* Western) and 26% (with *M. muricola* Eastern). According to Bradley and Baker (2006), and based on the variation in the mitochondrial DNA cytochrome *b* gene, the genetic values higher than 11% indicate species recognition. Therefore, there is genetically no doubt to recognise the taxonomic status of *M. muricola* in the Malay Archipelago as a distinct species from *M. mystacinus*.

Phylogenetic analysis revealed that *M. muricola*, which is widely distributed in the Malay Archipelago, consists of two major groups (namely, *M. muricola* Western and *M. muricola* Eastern), with a high genetic

distance between them. The average genetic distance between the two groups was 31.5%, and this ranged from 26.8% to 38.5% in the differences, suggesting species recognition. Within *M. muricola* Western, the population from Borneo was classified into a different sub-group and separated from *M. muricola* in Sumatra, Peninsular Malaysia and Asia mainland. The average genetic distance between the Bornean and Sumatra-Asian sub-groups was 7.2%, i.e. ranging from 5.1% to 10.8%. Based on these data, the two subgroups are genetically

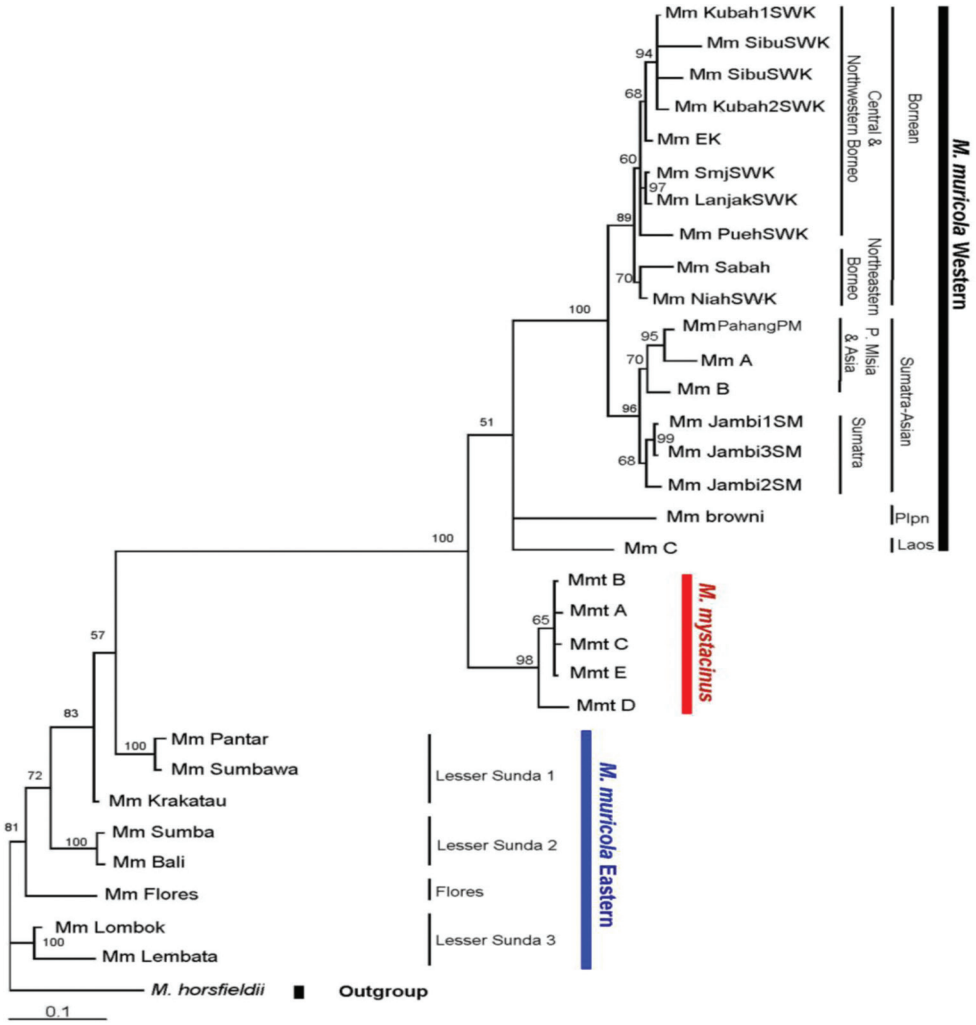

Fig. 6: The Bayesian phylogram showing the relationships between M. muricola *vs.* M. mystacinus *and between the groups and sub-groups within* M. muricola *populations based on the partial cytochrome b mtDNA as implemented in MR BAYES 3.0b4. The values on the branches represent Bayesian bootstrap values. Only the bootstrap values >50% are shown. Species abbreviations are as in Tables 1 and 2*

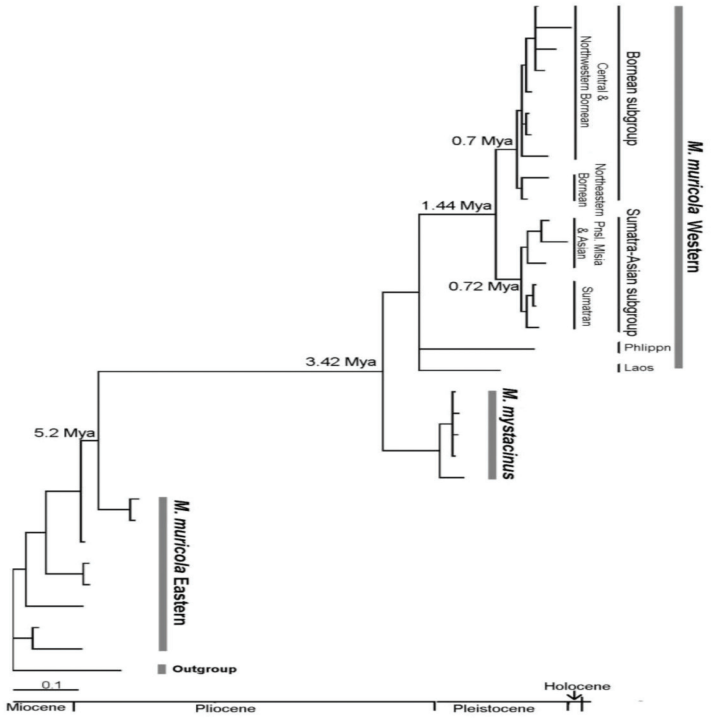

Fig. 7: A chronogram showing the estimated time of the divergence of M. muricola *and* M. mystacinus *obtained from the partial cytochrome b gene. Ages were inferred from the average genetic distance (Irwin et al., 1991) calculated using the Kimura two-parameter model (Kimura, 1980), implemented in MEGA version 3 (Kumar et al., 2004). The Bayesian phylogram is used to show the relationships among these populations*

recognised as different subspecies (Bradley & Baker, 2006). These genetic data become the evidence that the taxonomic status of this species must be resolved. Some new species and sub-species have also been recognised under *M. muricola*.

The Distribution Pattern of M. muricola *in the Malay Archipelago and the Implication of Wallace's Line as a Barrier*

On the basis of genetic, the populations from the larger islands of Greater Sunda (Sumatra and Borneo) and from Peninsular Malaysia formed one group, i.e. *M. muricola* Western. It is generally distinct from the islands in Lesser Sunda (Bali, Lombok, Lembata, Pantar, Flores, Sumba and Sumbawa). Meanwhile, the populations from Lesser Sunda, in combination with Krakatau, were clustered into one group, i.e. *M. muricola* Eastern. The phylogenetic analysis revealed that the two haplotypes from Bali and Krakatau were not clustered into *M. muricola* Western but into *M. muricola* Eastern instead. Geographically, the two localities are located on the west of the Wallace's Line region which is separated by the narrow Lombok Strait (about 23 km wide) as a barrier from the other islands in the east of the Wallace's Line region.

M. muricola Eastern had occurred during the Miocene (before early Pliocene) some 5.2 Mya. The drop in the sea level, however, had not only occurred over the last 2 Mya of the Earth history; the transitions from the early to middle Miocene and from the middle to the late Miocene were marked by the relatively low sea levels (Haq *et*

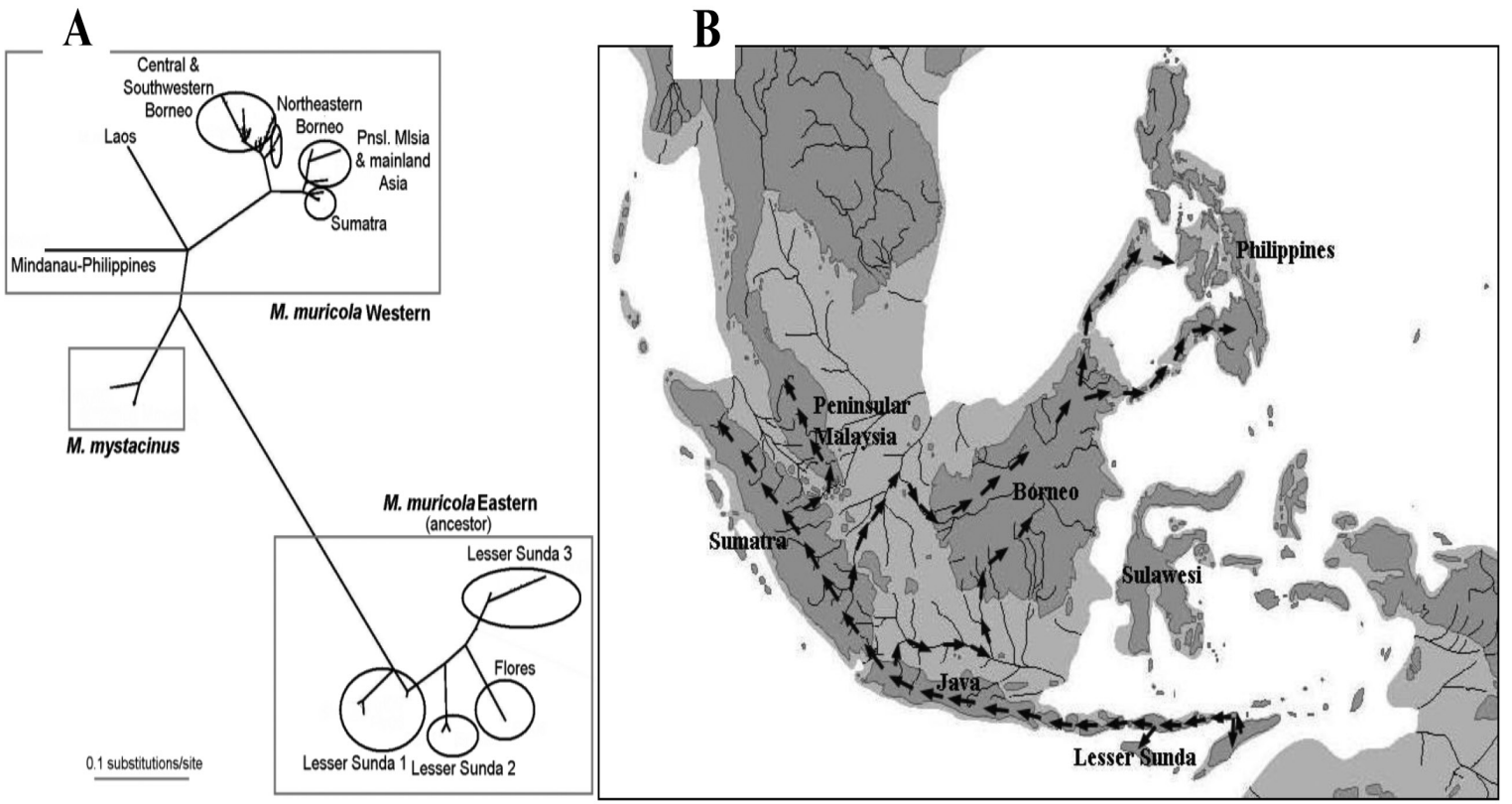

Fig. 8: The hypothetical routes (⟶) of the dispersal of M. muricola *in the Malay Archipelago.* ***A****: Radial tree showing the relationships between* M. muricola *and* M. mystacinus *and within M. muricola in the Malay Archipelago inferred from Bayesian method.* ***B****: Map showing the routes of the dispersal of M. muricola in the Malay Archipelago. Map was adapted or modified from Voris (2000), whereby the sea level was shown to drop 120 m below the present sea level giving rise to some river systems*

al., 1993), and this condition gave more chances for bats with their flight ability to cross the sea barrier, including Lombok Strait. The previous study on *Cynopterus titthaecheilus* showed little genetic differentiation between the bat populations in West Java (west of the Wallace's Line) and Lombok (east of the Wallace's Line) (Kitchener & Maharadatunkamsi, 1991). In addition, Kitchener and Maharadatunkamsi (1991) also noted that the water gap, such as those found between Bali and Lombok islands, did not pose a significant barrier to gene flow in other closely related *Cynopterus* species. Based on these data, this study has also indicated that the drop in the sea level during the Miocene (Haq *et al.,* 1993), as well as in the Lombok Strait, gave the possibility of gene flow within *M. muricola* Eastern, including those from the populations in Krakatau and Bali islands to the other populations in Lesser Sunda.

Sumatra, Borneo and Peninsular Malaysia are an expansion of the Asian continent and these places were connected to each other several times during the Pleistocene period when glaciations lowered the sea level (Heaney, 1991). Based on this genetic study, the samples of *M. muricola* from these islands were grouped into *M. muricola* Western. All the phylogenetic trees revealed that this particular group consisted of two sub-groups, namely, the Sumatra-Asian sub-group (Sumatra, Peninsular Malaysia and Asia mainland) and the Bornean sub-group (from Borneo only), which were supported with high bootstraps value (100% for NJ, MP, Bayesian and 99% for ML). The separation of the two sub-groups occurred during the Pleistocene, some 1.44 Mya. After the Pleistocene, these islands were separated by the Sea of Java and the populations inside these islands were isolated from one to the other island. This isolated population eventually appeared as a sister-group to all the other species in that particular group because any new mutation appearing outside the island did not reach the isolate and only the plesiomorphic characters were shared with the other areas (Ruedi & Fumagali, 1996). Meanwhile, the separation between the two sub-groups within *M. muricola* Western indicated allopatric speciation.

On the other hand, *M. muricola* Eastern has the higher genetic variation compared to the *M. muricola* Western. Based on the phylogenetic trees, members of this particular group did not form one cluster (clade) and thus had high genetic distances ranging from 1% to 16.4% in the difference. *M. muricola* Eastern consists of many islands from Lesser Sunda that were isolated from one another by sea or straits. Furthermore, the minimum contemporary sea crossing between the islands within the Eastern group ranged from four kilometres (between Flores and Lembata) to 117 kilometres (between Bali and Timor) (Hisheh *et al*., 2004). This condition might have led to the genetic differentiation among the populations within this group.

The Origin and Dating of the Diversification of M. muricola

The molecular analyses provide evidence on how the biogeography of *M. muricola* populations in the Malay Archipelago relates to their differentiation and diversification, and the relative timing of these events. Meanwhile, the genetic analyses support the monophyly of *M. muricola* and indicate that the common ancestor of this particular species in the Malay Archipelago is the population in the Lesser Sunda region, i.e. *M. muricola* Eastern. The position of this group, *M. muricola* Eastern, at the basal clade had started during the Miocene and it began to disperse to the western part of the Malay Archipelago during the Pliocene, some 5.2 Mya (*Fig. 7*). Although the sample from Krakatau Island was grouped into the Eastern group, it gave raise to the question in this study and became anomalous. According to Tidemann *et al.* (1990), after the cataclysmic eruption of 1883, the first bat colonisation found on this island some 20-30 years later was by Pteropodids. However, the Microchiropteran had more recent arrivals, probably during recolonising Rakata, i.e. between 50 and 70 years after the eruption. It is predicted that *M.*

muricola from this island are not the ancestor of *M. muricola* in the Malay Archipelago.

Ruedi and Mayer (2001) considered the extent to which paleontological and molecular data could be combined to infer the biogeographic patterns of evolution. The most recent interpretation of the fossil record supports a late Oligocene origin of the genus *Myotis* in Eurasia (Horacek, 2001), and the early appearance of the genus was followed in the early Miocene by burst of diversification, as indicated by the various fossils of *Myotis* found in Europe (Czaplewski, 1991). The evidence of *Myotis* fossils in Asia is fragmentary and more recent (late Pliocene/early Pleistocene), and based on the geographical reconstruction by Teeling *et al.* (2005), it was suggested that the bats originated from the Laurasian land masses which were possibly in North America during the early Paleocene. Nonetheless, the results from this study showed that the common ancestor of *M. muricola* was from Lesser Sunda Island in the Southeast Asia region, and this took place before the Pliocene. Thus, it is proposed that the findings of this study support the Gondwanan origin for the bats with the fossil of *Australonycteris* from Southeastern Queensland, as the evidence suggested that modern bats might have originated from an isolated group of archaic bats in the Southern Hemisphere (Hand, 1994) during the early Eocene. On the other hand, the geological history has also revealed that the animals could have reached the Malay Archipelago from Gondwanaland via Australia during the Miocene (Whitmore, 1981).

M. muricola Eastern diverged to the western part of the Malay Archipelago during the late Miocene to Pliocene, some 5.2 Mya. Cooling and aridification of habitats that occurred during the late Miocene (Cerling *et al.,* 1997) could have triggered the early diversification of bats. In the late Pliocene (some 3.4 Mya), *M. muricola* Eastern diverged into *M. muricola* Western. The genetic variations have proven that *M. muricola* Western is distinct from the common ancestor (i.e. *M. muricola* Eastern) and should be considered as a different species. During the Pleistocene (some 1.4 Mya), *M. muricola* Western was differentiated into two sub-groups, namely, the Sumatra-Asian and the Bornean sub-groups. During the glacial maximum, i.e. when the sea level was lowered to 120 m below that of the present, all the land areas of Sundaland were connected into one large land mass (Verstappen, 1975; Voris, 2000; Bird *et al*., 2005). At that time, many species could freely move through the moist ancient river systems that provided forest corridors in Sundaland, Sumatra, Peninsular Malaysia, Java and Borneo (Heaney, 1985; Voris, 2000).

Surprisingly, the present genetic study has suggested the sister relationship between *M. muricola* Western and *M. mystacinus*. Some of the previous studies have recorded that *M. mystacinus* is distributed from Europe through Nepal and India (Corbet & Hill, 1982; Francis, 2008), whereas Simmons (2005) noted that this particular species was distributed in Ireland and Scandinavia to Russia and the Ural Moutains, Kazakhstan, south of Syria, Israel, and Morocco, but not in Southeast Asia. On the other hand, the recent record of this particular species in Myanmar by Bates *et al*. (2005) is the first authenticated record from Southeast Asia. This study has indicated that *M. mystacinus* is also distributed in the Western part of the Malay Archipelago and this is sympatric with *M. muricola* Western.

These results provide the evidence for the evolution in *M. muricola*, including the origin and time of divergence. Meanwhile, a careful combination of climatic, fossil and genetic evidence can provide a much clearer picture of the evolution (Hewitt, 2000). However, the lack of samples is the limitation to further analysis. Moreover, complete molecular data sets and fossil data are needed for higher level analysis of the evolution of *M. muricola* to confirm the findings of this study.

Hypothetical Route of the Diversification and Distribution of M. muricola *in the Malay Archipelago*

The hypothetical route of colonisation and the diversification of *M. muricola* started from the common early ancestor in Lesser Sunda (*Fig. 8*). Based on the phylogenetic analysis, *M. muricola* Eastern at the basal clade has been predicted as the common ancestor of *M. muricola* in the Malay Archipelago, which later diverged into the western part of this region during the late Miocene to the early Pliocene. The origin of the ancestor was predicted to be either in Lombok or Lembata Island. The drop in the sea level at that time gave a chance to disperse from this origin to the western part of the Malay Archipelago.

Lesser Sunda was formed by the plate collision between Southeast Asia and Australia about 15 Mya (Hutchison, 1989). The habitat in the eastern part of Lesser Sunda was more arid than its western neighbour and it became unsuitable for some species to live in or survive (Mayr, 1944; Earl of Cranbrook, 2009). At that time, *M. muricola* Eastern migrated westward and reached the Java Island. Otherwise, the other evidence of the pigmy stegodonts (*Stegodon sompeonis*) from Java showed that this particular species had migrated from Laurasia to the Gondwanaland margin in Timor by walking on dry land along the volcanic island arch of Flores-Wetar (Audley-Charles, 1981). This evidence gave rise to the possibility of *M. muricola* Eastern, with flight ability for island hopping to reach Java Island through the opposite migration route of the pigmy stegodont.

Close to the Pleistocene (some 1.4 Mya), *M. muricola* colonised and diversified in Sumatra and continued into Peninsular Malaysia and Borneo. The estimation showed that the Pliocene-Pleistocene epoch was the period when the *M. muricola* species complex in Sundaland might have diversified. During the Pleistocene epoch, it was also observed that *M. muricola* divergence is similar to the diversification of the *Kerivoula* species in Sundaland (Faisal, 2008). The result of this study is also in a good agreement with the finding on other taxa by Inger and Voris (2001) who reported that the cladogenic speciation of frogs and snakes occurred in Sundaland over 1 Mya. The lowering of the sea level during the Pleistocene produced a number of land bridges. Sumatra, Java and Borneo were intermittently connected by the exposed Sunda Shelf. Some ancient river systems at that time served as the routes for migration (Voris, 2000; Bird *et al.*, 2005; Earl of Cranbrook, 2009), and possibly as the Pleistocene refuges of fauna as well. *M. muricola* diverged from Java to the Asian mainland through the Sumatra Island. Java was connected to Sumatra when the sea level dropped 50 m below the present sea level (Bird *et al.,* 2005). From Sumatra, *M. muricola* species complex diverged to Peninsular Malaysia through the Malacca Strait River System that connected these two localities. On the other hand, *M. muricola* in Borneo was suggested to have been migrated from Java through the two routes. Firstly, from Java, this species diverged into Sumatra and then reached Borneo through the North Sunda River System (Voris, 2000) via Bangka, Belitung and Karimata islands. The second route was through the East Sunda River System (Voris, 2000) which connected the northern coast of Java Island to the south coast of Borneo.

Meanwhile, the zoogeographic evidence suggested that at the Pliocene and early Pleistocene, there was a land bridge connecting the Philippines to Borneo (Heaney, 1985). This study speculated that this land bridge would be the probable route for *M. muricola* to colonise the Philippines, and thereafter, the population became isolated and adapted to the new habitat resulting it to diverge into *M. muricola browni*.

The geological history and the condition of the ecosystem are among the factors influencing the distribution patterns, as well as the morphological and genetic variations of the widespread species (Mayr, 1976). There was an increase in the climatic oscillations during the Pleistocene in all parts of the world, and thus, it was necessary to colonise, adapt or go extinct; the present lineages had the ability and luck to survive such environmental shifts (Hewitt, 2000). During the Pleistocene, Java Island was

divided into two vegetation types. East Java had more open vegetation type compared to West Java which was dominated by savanna (Bird *et al.,* 2005). Based on these data, it was speculated in this study that some populations of *M. muricola* in East Java had moved and colonised westwards, while the remaining populations adapted to these conditions.

The Bornean *M. muricola* subgroup diverged into two populations, namely, Central and Northwestern Borneo and Northeastern Borneo during the Pleistocene (*Fig. 5.1* and *5.2*). From this evidence, it was speculated that the habitat conditions at that time influenced the genetic variations within the Bornean sub-group. Among other, Meijard (2003) suggested that the north-western part of Borneo remained forested even during the last glacial period which could be suitable sites as Pleistocene refugia for fauna. Earl of Cranbrook (2000) postulated several areas as the probable Pleistocene refuges for the fauna in Northern Borneo, whereas Eastern Borneo was covered with open vegetation types like tree savanna or open deciduous forest (Meijard, 2003).

During the post Pleistocene geographical conditions, the present sea level gave raise to a lot of physical barriers for the fauna to easily migrate to the other islands. Moreover, there were no land bridges connecting the islands within the Sundaland region and within Lesser Sunda Islands. Thus, it was suggested that the barrier had caused geographical and reproductive isolations as well as gene flow obstacles that further led to allopatric speciation. Therefore, the recent study recorded the morphological and genetic variations of the recent populations of *M. muricola* that are related to the biogeography of this particular species in the Malay Archipelago.

CONCLUSION

Through the genetic analysis, *M. muricola* in the Malay Archipelago has been found to have high biogeographical variations and represent a species complex. Some new species and sub-species have been identified as well. Therefore, *M. muricola* Western and *M. muricola* Eastern should be recognised as two distinct species. Furthermore, the Sumatra-Asian and the Bornean sub-groups represent two different subspecies. In particular, *M. muricola* in the Malay Archipelago is monophyletic with *M. muricola* Eastern at the basal clade that has been hypothesised as the ancestor. Based on the molecular data, *M. muricola* Eastern had started to diverse into the western region during the Pliocene and diverged within the western region during the Pleistocene under the influence of the ancient river systems that had produced gallery forest corridors which could be considered as the Pleistocene refuges. Therefore, the results of this study have indicated that the revision of the taxonomic status and the re-evaluation of the evolutionary and biogeography of *M. muricola* are urgently needed. In addition, the lack of samples limited the analysis carried out in this study. Therefore, complete molecular data, using variable markers and representative samples from throughout the geographic range (specifically from Java and Lesser Sunda) are recommended for further studies so as to get the results firmly established.

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REFERENCES

Audley-Charles, M. G. (1981). Geological history of the region Wallace's Line. In T. C. Whitmore (Ed.). *Wallace's line and Plate Tectonic* (p. 91). Oxford: Clarendon Press.

Avise, J. C. (1998). The history and purview of phylogeography: a personal reflection. *Molecular Ecology*, *7*, 371-379.

Avise, J. C. (2000). *Phylogeography. The History and Formation of Species*. Cambridge: Harvard University Press.

Baker, R. J., & Bradley, R. D. (2006). Speciation in mammals and the Genetic Species Concept. *Journal of Mammalogy*, *87*(4), 643-662.

Bates, P. J. J., Hendrichsen, D. K., Walston, J. L., & Hayes, B. (1999). A review of the mouse-eared bats (Chiroptera: Vespertilionidae: *Myotis*) from Vietnam with significant new records. *Acta Chiropterologica*, *1*(1), 47-74.

Bates, P. J. J., Nwe, T., Bu, S. S. H., Mie, K. M., Swe, K. M., Nyo, N., Aye, A. K., et al. (2005). A review of the genera *Myotis, Ia, Pipistrellus*, *Hypsugo*, and *Arielulus* (Chiroptera: Vespertilionidae) from Myanmar (Burma), including three species new to the country. *Acta Chiropterologica, 7*(2), 205-236.

Bird, M. I., Taylor, D., & Hunt, C. (2005). Palaeoenvironments of insular Southeast Asia during the last Glacial Period: a savanna corridor in Sundaland? *Quarternary Science Reviews*, *24*, 2228-2242.

Cerling, T. E., Harris, J. M., MacFadden, B. J., Leakey, M. L., Quade, J., Eisenmann, V., & Ehleringer, J. R. (1997). Global vegetation change through the Miocene/Pliocene boundary. *Nature*, *389*, 153-158.

Chasen, F. N. (1940). A Handlist of Malaysian Mammals. *Bulletin of the Raffles Museum*, *15,* 1-209.

Corbet, G. B. (1978). *The mammals of the Palearctic region. A taxonomic review*. London: Intl Specialized Book Service Inc.

Corbet, G. B., & Hill, J. E. (1992). *The Mammals of the Indomalayan Region: a systematic review*. London: British Museum (Natural History).

Czaplewski, N. J. (1991). Miocene bats from the lower Valentine Formation of northeastern Nebraske. *Journal of Mammalogy*, *72*, 715-722.

Davalos, L. M., & Perkins, S. L. (2008). Saturation and base composition bias explain phylogenomic conflict. *Plasmodium*. *Genomic*, *91*, 433-442.

Di Mito, C., & Betschart, B. (1998). Biomedical and life sciences. *Parasitology Research*, *84*(7): 596-597.

Ducroz, J. F., Volobouv, V., & Granjon, L. (1998). A molecular perspective systematic and evolution of the Genus *Arvicanthis* (Rodentia: Muridae): Inferences from complete cytochrome *b* gene Sequences. *Molecular Phylogenetics and Evolution*, *10*(1), 104-117.

Earl of Cranbrook, E. o. C. (2000). Quarternary mammal fossils from Borneo. *Sarawak Museum Journal*, *55*(76), 216-233.

Earl of Cranbrook, E. o. C. (2010). Late quaternary turnover of mammals in Borneo: the zooarchaeological record. *Biodiversity and Conservation, 19*(2), 373-391.

Faisal, A. K. (2008). *Diversification of Old World Bats in Malaysia: An Evolutionary and Phylogeography. Hypothesis tested through Genetic Species Concept*. (MSc Thesis dissertaion). Texas Tech University, Lubbock, US.

Felsenstein, J. (1988). Phylogenies from molecular sequences: Inference and reliability. *Annual Review on Genetic*, *22*, 521-565.

Francis, C. M. (2008). *A Field Guide to The Mammal of Southeast Asia*. London: New Holland Publisher.

Funk, D. J., & Omland, K. E. (2003). Species-level Paraphyly and Polyphyly: frequency, causes and consequences, with insight from animal mitochondrial DNA. *Annual Review of Ecogical, Evolution and Systematic*, *34*, 397-423.

Galtier, N., Nabholz, B., Glemin, S., & Hurst, G. D. D. (2009). Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology*, *18*, 4541-4550.

Garcia-Mudarra, J. L., Ibanez, C., & Juste, J. (2009). The Straits of Gibraltar: barrier or bridge to

Ibero-moroccan bat diversity?. *Biological Journal of the Linnean Society. London*, *96*, 434-450.

Gari, M. A., Abuzenadah, A. M., Chaudhary, A. G., Al-Qahtani, M. H., Al-Says, F. M., & Dmanhouri, G. (2006). Pilot study of DNA extraction. *African Journal of Biotechnology*, *5*(6), 532-535.

Hand, S. J., Novacek, M. J., Godthelp, H., & Archer, M. (1994). First Eocene bat from Australia. *Journal of Vertebrate Paleontology*, *14*, 375-381.

Haq, B. U., Hardenbol, J., & Vail, P. R. (1993). Chronology of fluctuating sea levels since the Triassic. *Science*, *235*, 1156-1167.

Hasan, N. H. (2008). *A Morphological And Molecular Phylogenetic Analysis of Malaysian Kerivoula (Chiroptera: Vespertilionidae)*. (MSc thesis dissertation). Universiti Malaysia Sarawak, Sarawak, Malaysia.

Heaney, L. R. (1985). Zoogeographic evidence for middle and late Pleistocene land bridges to the Philippine Islands. *Modern Quarternary Research in Southeast Asia*, *9*, 127-143.

Heaney, L. R. (1991). A synopsis of climatic and vegetational changes in Southeast Asia. *Climatic Change*, *19*, 53-61.

Hewitt, G. (2000). The genetic legacy of the Quarternary ice ages. *Nature*, *405*(6789), 907-913.

Hisheh, S., How, R. A, Suyanto, A., & Schmitt, L. H. (2004). Implications of contrasting of genetic variability in two vespertilionid bats from Indonesian Archipelago. *Biological Journal of the Linnean Society*, *83*, 421-431.

Hisheh, S., Westerman, M., & Schmitt, L. H. (1998). Biogeography of the Indonesian Archipelago: mitochondrial DNA variation in the fruit bat, *Eonycteris spelaea. Biological Journal of the Linnean Society*, *65*, 329-345.

Horaceck, I. (2001). On the early history of vespertilionid bats in Europe: the Lower Miocene record from the Bohemian Massif. *Lynx*, *33*, 123-154.

Hutchison, C. S. (1989). *Geological Evolution of Southeast Asia*. Oxford: Oxford University Press.

Ibanez, C., Garcia-Mudarra, J. L., Ruedi, M., Stadelmann, B., & Juste, J. (2006). The Iberian contribution to cryptic diverdity in European bats. *Acta Chiropterologica*, *8*(2), 277-297.

Inger, R. H., & Voris, H. K. (2001). The biogeographical relations of the frog and snakes of Sundaland. *Journal of Biogeography*, *28*, 863-891.

Irwin, D. M., Koecher, T. D., & Wilson, A. C. (1991). Evolution of the cytochrome *b* gene of mammals. *Journal of Molecular Evolution*, *32*, 128-144.

Jayaraj, V. K. (2008). *The Phylogenetic Relationships of Megachiroptera in Malaysia Inferred From Morphological and DNA Analysis*. (MSc thesis dissertation). Universiti Malaysia Sarawak, Sawawak, Malaysia.

Kawai, K. Nikaido, M., Harade, M., Matsumara, S., Lin, L. K., Wu, Y., Hasegawa, M., & Okada, N. (2003). The status of Japanese and East Asia Bats of the genus *Myotis* (Vespertilionidae) based on mitochondrial sequence. *Molecular Phylogenetics and Evolutions*, *28*, 297-307.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*, 111-120.

Kimura, M. (1981). Estimation of evolutionary distances between homologous nucleotide sequences. *Proceeding of National Acedemy of Sciences. USA*, *78*, 454-458.

Kimura, M. (1981). Estimation of evolutionary distances between homologous nucleotide sequences. *Proceeding of National Acedemy of Sciences. USA*, 78, 454-458.

Kitchener, D. J., & Maharadatunkamsi. (1991). Description of a new species of *Cynopterus* (Chiroptera: Pteropodidae) from Nusa Tenggara, Indonesia. *Record of the Western Australian Museum, 15*, 307-363.

Kumar, S., Tamura, K., & Nei, M. (2004). MEGA 3: Integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. *Briefings in Bioinformatics*, *5*, 150-163.

Laurie, E. M. O., & Hill, J. E. (1954*). List of land mammals of New Guinea, Celebes and adjacent islands 1758-1952*. London: British Museum Natural History.

Lecompte, E., Denys, C., & Granjon, L. (2005). Confrontation of morphological and molecular

data: The *Promys* group (Rodentia: Murinae) as a case of adaptive convergences and morphological stasis. *Molecular Phylogenetics and Evolution*, *37*, 899-919.

Maharadatunkamsi, Hisheh, S., Kitchener, D. J., & Shmitt, L. (2000). Genetic and morphometric diversity in Wallacea: geographical patterning in the horse-shoe bat, *Rhinolophus affinis*. *Journal of Biogeography, 27*, 193-201.

Mayr, E. (1976). *Evolution And The Diversity of Life*. Cambridge: Belknap Press.

McCharty, C. (1996). *CHROMAS version 1.45 program*. Schools of Health Science, Griffith University, Queensland, Australia.

Meijaard, E. (2003). Mammals of Southeast Asian islands and their late Pleistocene environments. *Journal of Biogeography*, *30*, 1245-1257.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A. B., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*, 853-858.

Nylander, J. A. A. (2004). *MrAIC.pl*, Uppsala: Evolutionary Biology Center, Uppsala University.

Oldemann, L. R., Irsal, L., & Muladi. (1980). The agroclimatic maps of Kalimantan, Maluku, Irian Jaya and Bali, West and East Nusa Tenggara. *Contribution for the Central Research Institute for Agriculture, Bogor*, *60*, 1-32.

Palumbi, S. R., Martin, Romano, S., Mcmillan, W. O., Stice, L., & Grabowski, G. (1991). *The Simple Fools's Guide to PCR*. Honolulu: Department of Zoology and Kewalo Maine Laboratory, University of Hawaii.

Piaggio, A. J., Valdez, E. W., Bogan, M. A., & Spicer, G. S. (2002). Systematic of *Myotis occultus* (Chiroptera: Vespertilionidae) inferred from sequences of two mitochondrial genes. *Journal of Mammalogy*, *83*, 386-395.

Posada, D., & Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics, 14*, 817-818.

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, *19*, 1572-1574.

Ruedi, M., & Fumagali, L. (1996). Genetic structure of Gymnures (genus *Hylomys*: Erinaceidae) on continental island of Southeast Asia: Historical effects of fragmentation. *Journal Zoological Systematic and Evolution Research*, *34*, 153-162.

Ruedi, M., & Mayer, E. (2001). Molecular systematic of bats of the genus *Myotis* (Vespertilionidae) suggests deterministic ecomorphological convergences. *Molecular Phylogenetics and Evolution*, *21*, 436-448.

Saitou, N., & Nei, M. (1987). The Neighbor-joining Method: A New Method for Reconstructing Phylogenetic Trees. *Molecular Biology and Evolution*, *4*, 406-425.

Schmitt, L. H., Kitchener, D. J., & How, R. A. (1995). A genetic perspective of mammalian variation and evolution in the Indonesian archipelago: Biogeographic correlates in the fruit bat genus *Cynopterus*. *Evolution*, *49*(3), 339-412.

Simmons, N. B. (2005). Order Chiroptera. In Wilson, D. E., & Reeder. *Mammal Species of the World: A Taxonomic and Geographic Reference*. Baltimore: John Hopkins University Press.

Stadelmann, B., Lin, L. K., Kunz, T. H., & Ruedi, M. (2007). Molecular Phylogeny of New World *Myotis* (Chiroptera: Vespertilionidae) Inferred from Mitochondrial and Nuclear DNA genes. *Molecular Phylogenetics and Evolution*, *43*, 32-48.

Suyanto, A. (2001). *Kelelawar di Indonesia*. Bogor: Lembaga Ilmu Pengetahuan Indonesia.

Tate, G. H. H. (1941). Result of the Archbold Expedition No. 40: Notes on Vespertilionid bats. *Bulletin of the American Museum of Natural History*, *78*(10), 567-597.

Teeling, E., Springer, M. S.,Madsen, O., Bates, P., O'Brien, S. J., & Murphy, W. J. (2005). A molecular phylogeny for bats illuminates biogeography and the fossil record. *Science*, *307*, 580-584.

Thompson, J. D., Gibson, T. J., & Plewniak, F. (1997). The Clustal X Windows Interface: Flexible Strategies for Multiple Sequence Alignment Aided by the Quality Analysis Tools. *Nucleic Acids Research*, *24*, 4876-4882.

Tidemann, C. R., Kitchener, D. J., Zann, R. A., & Thornton, I. W. B. (1990). Recolonisation of the Krakatau Islands and Adjacent Areas of West Java, Indonesia by Bats (Chiroptera) 1883-1986. *Philosophical Transactions of The Royal Society London. B: Biological Science, 328*, 123-130.

Tsytsulina, K., Dick, M. L., Masuda, R., & Maeda, K. (2004). *Phylogeography and systematic of the steppe whiskered bat Myotis aurascens Kuzyakin, 1935 (Chiroptera, Vespertilionidae)*. Center for Advanced Science and Technology, Hokkaido University (unpublished).

van Strien, N. J. (1986). *Abbreviated checklist of the mammals of the Australian Archipelago*. Bogor: School of Environmental Conservation Management.

Verstappen, H. T. (1975). On palaeoclimates and landform development in Malesia. In: Bartstra, G. J., & Casparie, W. A. *Modern Quarternary Research in Southeast Asia*. Rotterdam: Taylor & Francis.

Voris, H. K. (2000). Maps of Pleistocene sea level in Southeast Asia: shorelines, river system and time durations. *Journal of Biogeography*, *27*, 1153-1167.

White, P. S., & Densmore, L. D. (1992). Mitochondrial DNA isolation. In: Hoelzel, A.R. (Ed). *Molecular genetic analysis of population*. Oxford: IRL Press.

Whitmore, T. C. (1987). *Biogeographical evolution of the Malay Archipelago*. Oxford: Clarendon Press.

Xia, X., & Xie, Z. (2001). DAMBE: Data analysis in molecular biology and evolution. *Journal of Heredity*, *92*, 371-373.

Yasuma, S., Andau, M., Apin, L., Yit Yu, F. T., & Kimsui, L. (2003). *Identification Key to The Mammals of Borneo*. Kota Kinabalu: Japan International Cooperation Agency-Sabah Park-Jabatan Hidupan Liar Sabah-Universiti Malaysia Sabah.