

Phylogenetic Relationships and Morphological Variation of Gymnures (Galericidae: *Hylomys*) from Genting Highlands, Pahang, Malaysia

(Hubungan Filogenetik dan Variasi Morfologi Tikus Babi (Galericidae: *Hylomys*) daripada Tanah Tinggi Genting, Pahang, Malaysia)

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ABSTRACT

Gymnures from the genus *Hylomys* are widely distributed across Sundaland and the Indochinese region. However, the relationships among its members in Peninsular Malaysia especially from Genting Highlands are still lacking. This study aims to examine the morphology and genetically analyse of the *Hylomys* specimens collected from the new isolated population of Genting Highlands, Pahang, Malaysia. Morphometric analyses showed that morphological variation exists among the *Hylomys* lineage. The external morphology and the skull measurements of the taxa studied coincide with those of *Hylomys maxi* from Peninsular Malaysia and Sumatra based on descriptive statistics and principal component analysis (PCA). The differences within the *Hylomys* taxa are also evident in the phylogenetic tree and K2P genetic distances analyses inferred from *cyt b*. *Hylomys* samples from Genting Highlands formed a clade with *H. maxi* from Cameron Highlands and Fraser's Hill, Peninsular Malaysia and Sumatra. However, they were separated from *Hylomys parvus* (Sumatra), *Hylomys suillus* (Java) and *Hylomys dorsalis* (Borneo). Relatively, low genetic distances were detected among the studied taxa with other *H. maxi* (<6% K2P distance) while high genetic distances were observed when compared to other *Hylomys* taxa (>18.0% K2P distance). Based on these facts, we confirm that the gymnures from Genting Highlands belong to a valid species, *Hylomys maxi* along with other Peninsular Malaysia samples based on genetics and morphological data which probably originated from Sumatra a long time ago.

Keywords: Biogeography; Eulipotyphyla; genetic relationships; morphology; Peninsular Malaysia

ABSTRAK

Tikus babi daripada genus *Hylomys* mempunyai taburan yang luas di Sundaland dan Indochina. Walau bagaimanapun, kajian mengenai hubungan antara kumpulan tikus babi di Semenanjung Malaysia terutamanya dari Tanah Tinggi Genting masih kurang dijalankan. Penyelidikan ini bertujuan untuk mengkaji morfologi dan menganalisis secara genetik spesimen *Hylomys* yang dikumpul daripada populasi baru Tanah Tinggi Genting, Pahang, Malaysia. Analisis morfometrik mendedahkan bahawa variasi morfologi wujud dalam kalangan *Hylomys*. Morfologi luaran dan ukuran tengkorak takson yang dikaji bertepatan dengan *Hylomys maxi* dari Semenanjung Malaysia dan Sumatera berdasarkan statistik deskriptif dan analisis komponen utama (PCA). Perbezaan dalam takson *Hylomys* juga jelas pada pokok filogenetik dan analisis jarak genetik K2P berdasarkan urutan gen *cyt b*. Sampel *Hylomys* dari Tanah Tinggi Genting membentuk klad dengan *H. maxi* dari Tanah Tinggi Cameron dan Bukit Fraser, Semenanjung Malaysia dan

Sumatera. Walau bagaimanapun, mereka dipisahkan daripada *Hylomys parvus* (Sumatera), *Hylomys suillus* (Jawa) dan *Hylomys dorsalis* (Borneo). Secara relatifnya, jarak genetik yang rendah dikesan dalam kalangan takson yang dikaji dengan *H. maxi* lain (<6% jarak K2P) manakala jarak genetik yang tinggi diperhatikan jika dibandingkan dengan takson *Hylomys* yang lain (>18.0% jarak K2P). Berdasarkan fakta ini, kami mengesahkan bahawa tikus babi dari Tanah Tinggi Genting tergolong dalam spesies yang sah, *Hylomys maxi* bersama sampel Semenanjung Malaysia yang lain berdasarkan data genetik dan morfologi yang mungkin berasal dari Sumatera pada satu masa dahulu.

Kata kunci: Biogeografi; Eulipotyphyla; hubungan genetik; morfologi; Semenanjung Malaysia

INTRODUCTION

According to the former taxonomic status, gymnures and moonrats belong to the distinct subfamily of Galericinae Pomel, 1848 while hedgehogs belong to the subfamily Erinaceinae Fisher, 1814 within the family Erinaceidae Fisher, 1814 (He et al. 2012; Hutterer 2005; Mckenna & Bell 1997). They were classified under the order of Eulipotyphla Waddell et al. 1999 and suborder Erinaceomorpha Gregory, 1910. However, based on the revision on this order in Bannikova et al. (2014), the subfamily Galericinae and Erinaceinae were treated as the family Galericiidae (gymnures and moonrats) and Erinaceidae (hedgehogs) respectively due to ancient origin, deep genetic divergence and morphological differentiation. Bannikova et al. (2014) also introduced a new tribe, Otohylomyini within the family Galericiidae along with two former tribes, Echinisoricini Cabrera, 1925 and Hylomyini Anderson, 1879.

Based on Corbet (1988), there are five genera within this Erinaceomorpha. However, Frost et al. (1991) validated only three genera which consist of *Echinorex* Raffles, 1822, *Podogymnura* Blainville, 1838 and *Hylomys* Müller, 1840 while *Neotetracus* Trouessart, 1909 and *Neohylomys* Shaw and Wong, 1959 belonged to the genus *Hylomys* due to insignificant cranial differences. Nevertheless, Bannikova et al. (2014) and Engesser and Jiang (2011) clarified the odontological, craniological variances and genetic divergence thus validated both genera and separated them from the genus *Hylomys*.

The genus *Hylomys* from the tribe Hylomyini ranges from lowland to highland moist jungle terrain with dense and thick undergrowth (Corbet & Hill 1992; Wilson & Mittermeier 2018). They are distributed in various locales of Southeast Asia such as major islands which includes Borneo, Sumatra and Java, Peninsular Malaysia as well as Indochina regions which include Thailand, Myanmar, Lao PDR, Cambodia, China and Vietnam (Corbet & Hill 1992; Medway 1983; Nor 2001; Ruedi & Fumagalli 1996; Smith et al. 2008; Wilson & Mittermeier 2018).

Due to extensive diversification, the taxonomic status of the genus *Hylomys* and its species underwent many taxonomic revisions. For instance, the long-eared gymnure from Laos which was initially known as *Hylomys megalotis* Jenkins and Robinson, 2002, is currently treated as a new genus, *Otohylomyis* Bannikova et al. 2014. It is now known as *Otohylomyis megalotis*, a monotypic species in the new tribe, Otohylomyini. This taxonomic revision leaves two species within the genus *Hylomys* which consist of *Hylomys suillus* Müller, 1840 also known as the short-tailed gymnure or lesser gymnure and *Hylomys parvus* Robinson and Kloss, 1916, the endemic species from Sumatra, Indonesia which is also known as the dwarf gymnure (Corbet & Hill 1992; Frost et al. 1991; Hutterer 2005; Wilson & Mittermeier 2018). *Hylomys suillus* has the widest distribution with several subspecies being identified in the past. Corbet and Hill (1992) proposed and recognized five subspecies: i) *H. s. dorsalis* Thomas, 1888 (Borneo), ii) *H. s. microtinus* Thomas, 1925 (Vietnam), iii) *H. s. maxi* Sody, 1933 (Sumatra), iv) *H. s. tionis* Chasen, 1940 (Tioman Island of Malay Peninsula) and v) *H. s. suillus* (Java, Sumatra and mainland). Recently, Wilson and Mittermeier (2018) recognized seven subspecies: i) *H. s. suillus* Müller, 1840 (Java), ii) *H. s. dorsalis* (Borneo), iii) *H. s. maxi* (Thailand, Malay Peninsula and Sumatra), iv) *H. s. microtinus* (China, Vietnam and Cambodia), v) *H. s. peguensis* (Myanmar and China), vi) *H. s. siamensis* Kloss, 1916 (Myanmar, Thailand and Laos) and vii) *H. s. tionis* (Tioman Island). According to Jenkins and Robinson (2002) and Ruedi et al. (1994), these high levels of variations could be due to geographical and altitudinal isolation.

The taxonomic status of these subspecies is still ambiguous, and some were re-evaluated by previous studies (Bannikova et al. 2014; He et al. 2012; Jenkins & Robinson 2002). These authors suggested a species level to some of these *Hylomys* subspecies due to genetic divergence of the mitochondrial cytochrome *b*. Therefore, in this study, we considered *H. s. maxi* as a valid species,

H. maxi along with other subspecies *H. s. suillus*, *H. s. dorsalis*, *H. s. siamensis*, and *H. s. microtinus* as species level, *H. suillus*, *H. dorsalis*, *H. siamensis* and *H. microtinus*, respectively. However, we maintained their subspecies ranks of two *Hylomys* subspecies, *H. s. peguensis* and *H. s. tionis* due to unavailable data and lack of studies.

In accordance with the latest taxonomic revision by these authors (Bannikova et al. 2014; He et al. 2012; Jenkins & Robinson 2002), the *Hylomys* species that have been recognized in Sundaland are *H. suillus* from Java, *H. maxi* from Peninsular Malaysia and Sumatra, *H. dorsalis* from Borneo and the *H. parvus* endemic in Sumatra. During the last two decades, numerous studies from various sampling localities for gymnures from Peninsular Malaysia were conducted. These include the study of genetic structure as well as biochemical and morphological analyses for samples from Cameron Highlands and Fraser's Hill, Pahang (Ruedi & Fumagalli 1996; Ruedi et al. 1994), the biodiversity inventory of *H. maxi* samples from Kedah Peak, Kedah (Langham 1983) and Mount Nuang, Hulu Langat, Selangor (Batin et al. 2002) as well as *H. s. tionis* from Tioman Island, Johor in Peninsular Malaysia (Lim et al. 1999). Despite that, there is a scarcity of comprehensive studies conducted on phylogenetic relationships of *Hylomys* species from Peninsular Malaysia.

Previously, the *Hylomys* species was only found in Cameron Highlands and Fraser's Hill from the highland of Pahang, Malaysia (Ruedi et al. 1994). Our recent survey led to the new collection of a few gymnure samples representing the third isolated population of the species from Genting Highlands, Pahang. Therefore, in this study, the taxonomic revision of the gymnures has been done by investigating the phylogenetic relationship of the *Hylomys* species from Peninsular Malaysia (particularly Genting Highlands samples) with other *Hylomys* taxa and related species from Sundaland and Indochina. With additional samples collected from the highland of Peninsular Malaysia, we analysed the morphological measurements of *Hylomys* to get an overview of morphological variations of this genus in Sundaland. We will also generally discuss the dispersal history of this genus.

MATERIALS AND METHODS

SAMPLE COLLECTION

The *Hylomys* samples were collected at Genting Highlands, Peninsular Malaysia (Figure 1) using 100 cage traps sized 28 cm × 15 cm × 12 cm baited with salted

dried fish. The trapped *Hylomys* were then euthanized in a sealed container connected to a carbon dioxide (CO₂) tank. The external measurements and sex were immediately recorded. Liver samples were taken as they yielded higher quality and concentration of DNA which will be preserved in 90% ethanol and stored at -40 °C for molecular studies. Specimens were either preserved in 70% alcohol or catalogued and deposited in the Museum of Zoology, Universiti Malaya (MZUM) for future references. The extracted skulls of *Hylomys* were preserved separately for morphological study. The museum skull specimens of the *Hylomys* species from the Museum of Zoology, Universiti Malaya (MZUM) and Lee Kong Chian Natural History Museum, Singapore (ZRC) were also measured and included for comparative studies. The museum specimen numbers and localities are listed in Appendix A.

MORPHOLOGICAL ANALYSES

Following Omar et al. (2013) and Ruedi et al. (1994), the external morphology of the *Hylomys* samples were measured and recorded (Table 1). Sixteen cranial characters from the preserved skulls were studied (Table 2) based on Ruedi et al. (1994). All the samples were classified as adults based on body weights, dental attritions and development in genital organs (Arai et al. 1985). To compare the skull characters of the seven samples captured from Genting Highlands along with 35 museum specimens of *Hylomys*, we computed the descriptive statistics and performed principal component analysis (PCA) using R version 4.0.2. The ANOVA results based on their external and skull measurements showed that sexual dimorphism was absent. The descriptive statistics of the external morphology and skull characters were also calculated (Tables 1 & 2). The external measurements of other *Hylomys* species were taken from Ruedi et al. (1994) for comparative studies (Table 1).

PCR AMPLIFICATION AND DNA SEQUENCING

The DNA was extracted from 90% ethanol-preserved liver samples using GF-1 Tissue DNA nucleic acid extraction kits (Vivantis, USA) as the liver samples' extraction will provide high quality DNA. These DNAs were amplified by polymerase chain reaction (PCR) of the mitochondrial cytochrome *b* (*cyt b*) gene using universal primer pairs L14734 and H15985 (Ohdachi et al. 2001). 25 µL PCR reactions contain 1 µL of DNA template, 1 µL of each primer (10 mM), 12.5 PCR Master mix and 9.5 µL of ultrapure water (UPW). The PCR

conditions are as follows: 35 cycles of denaturation at 94 °C for 30 s, annealing at 46.8 °C for 30 s, extension at 72 °C for 1 min, with a final extension at 72 °C for

6 min. PCR products that are verified with successful amplification via electrophoreses were sequenced by Mytagc Bioscience Enterprise, Selangor, Malaysia.

TABLE 1. Comparison of external measurements of *Hylomys* lineages. Mean \pm standard deviation (SD) and range (in parenthesis) for each taxon. Sample size is given in parentheses adjacent to SD. All lengths are in millimetres (mm) and weights, in grams (g). The measurements of other species (previously treated as *suillus* subspecies except for *H. parvus*) were taken from Ruedi et al. (1994).

Species	<i>Hylomys</i> sp. (Genting Highlands)	<i>H. maxi</i> (Peninsular Malaysia & Sumatra)	<i>H. suillus</i> (Java)	<i>H. dorsalis</i> (Borneo)	<i>H. parvus</i> (Sumatra)
External Morphology					
Head to Body (HB)	133.4 \pm 7.2 (7) (125 – 148)	135.7 \pm 9.4 (21) (122 – 156)	130.9 \pm 8.5 (41) (111 – 152)	135.9 \pm 7.7 (8) (122 – 145)	108.5 \pm 7.0 (15) (100 – 127)
Tail length (TL)	13.0 \pm 2.7 (7) (8 – 16)	14.6 \pm 6.4 (11) (3 – 24)	18.5 \pm 4.0 (36) (12 – 26)	19.8 \pm 7.6 (3) (13 – 26)	23.1 \pm 5.4 (14) (15 – 32)
Hind foot length without claw (HF)	25.9 \pm 1.2 (7) (24 – 28)	24.6 \pm 1.3 (21) (23 – 36)	24.4 \pm 1.7 (41) (20 – 28)	25.0 \pm 2.0 (8) (21 – 28)	23.0 \pm 0.8 (16) (22 – 25)
Ear Length (E)	15.6 \pm 1.1 (7) (14 – 17)	17.1 \pm 2.3 (20) (14 – 22)	16.6 \pm 1.4 (41) (13 – 21)	17.4 \pm 1.2 (7) (15.5 – 18.7)	16.5 \pm 1.3 (16) (14 – 19)
Weight (W)	65.7 \pm 12.0 (7) (43 – 80)	–	–	–	–

TABLE 2. Comparison of skull measurements among *Hylomys* lineages. The *Hylomys* taxa measurements (except from Genting Highlands) were collected from museum samples. Sample size (*n*) followed by mean \pm standard deviation and range (in parenthesis) for each taxon. All measurements are in millimetres (mm)

Species	<i>Hylomys</i> sp. (Genting Highlands) (n = 7)	<i>H. maxi</i> (Peninsular Malaysia) (n = 10)	<i>H. suillus</i> (Java) (n = 5)	<i>H. dorsalis</i> (Borneo) (n = 10)	<i>H. parvus</i> (Sumatra) (n = 4)
Skull Characters					
Greatest length of skull (GLS)	36.7 \pm 0.3 (36.2 – 37.1)	36.7 \pm 0.6 (35.9 – 37.6)	33.8 \pm 0.6 (33.0 – 34.6)	36.6 \pm 0.8 (35.5 – 37.9)	30.6 \pm 1.9 (29.2 – 33.4)
Condylbasal length (CBL)	35.2 \pm 0.7 (34.1 – 36.2)	35.0 \pm 0.7 (34.2 – 36.4)	31.7 \pm 0.4 (31.1 – 32.1)	35.6 \pm 0.9 (34.0 – 36.9)	29.4 \pm 2.2 (27.2 – 32.3)
Braincase breadth (BB)	14.8 \pm 0.4 (14.4 – 15.3)	14.7 \pm 0.4 (14.0 – 15.2)	14.8 \pm 0.6 (14.2 – 15.5)	15.2 \pm 0.3 (14.8 – 15.8)	13.4 \pm 0.3 (13.2 – 13.8)
Interorbital breadth (IOB)	8.2 \pm 0.2 (8.0 – 8.5)	7.9 \pm 0.5 (6.9 – 8.5)	9.4 \pm 0.3 (8.9 – 9.9)	9.6 \pm 0.5 (9.1 – 10.4)	7.6 \pm 0.6 (7.2 – 8.3)

Rostral length (ROL)	16.8 ± 0.2 (16.5 – 17.1)	16.9 ± 0.7 (15.2 – 17.5)	15.7 ± 0.7 (15.0 – 16.7)	17.6 ± 0.6 (16.8 – 18.5)	14.1 ± 1.0 (12.6 – 14.8)
Rostral breadth (ROB)	6.5 ± 0.2 (6.3 – 6.8)	6.4 ± 0.3 (6.0 – 6.9)	6.0 ± 0.4 (5.6 – 6.8)	5.9 ± 0.5 (5.4 – 6.8)	5.1 ± 0.4 (4.6 – 5.7)
Postpalatal length (PPL)	13.5 ± 0.4 (13.1 – 14.1)	14.1 ± 0.6 (12.9 – 15.0)	11.9 ± 0.5 (11.4 – 12.6)	13.4 ± 0.7 (12.5 – 14.7)	10.7 ± 0.7 (10.1 – 11.6)
Postpalatal depth (PPD)	9.3 ± 0.2 (9.1 – 9.6)	9.1 ± 0.3 (8.7 – 9.5)	8.9 ± 0.5 (8.3 – 9.6)	9.1 ± 0.4 (8.6 – 9.9)	7.9 ± 0.6 (7.1 – 8.7)
Breadth at third molars (M3B)	6.6 ± 0.3 (5.9 – 7.0)	6.4 ± 0.3 (5.9 – 7.0)	6.9 ± 0.7 (6.2 – 7.9)	6.8 ± 0.7 (6.0 – 7.8)	5.7 ± 0.6 (5.3 – 6.6)
Length of upper molariform (PM3)	8.8 ± 0.4 (8.5 – 9.5)	8.8 ± 0.5 (7.8 – 9.5)	10.3 ± 0.5 (9.6 – 10.9)	8.4 ± 0.5 (8.1 – 9.3)	9.1 ± 2.0 (6.2 – 10.7)
Length of upper tooth row (IM3S)	19.2 ± 0.5 (18.4 – 20.0)	18.9 ± 0.5 (17.8 – 19.5)	17.7 ± 0.7 (16.9 – 18.7)	18.7 ± 0.5 (18.1 – 19.6)	15.6 ± 1.1 (14.4 – 16.8)
P ⁴ to M ³ length (P4M3)	9.8 ± 0.3 (9.3 – 10.2)	9.6 ± 0.3 (9.0 – 10.0)	8.7 ± 0.6 (8.1 – 9.4)	8.7 ± 0.5 (8.4 – 9.6)	7.9 ± 0.3 (7.5 – 8.2)
Mandibular length (MAL)	24.9 ± 0.5 (24.1 – 25.5)	24.5 ± 0.8 (22.7 – 25.5)	23.9 ± 0.5 (23.4 – 24.6)	24.5 ± 0.9 (23.4 – 25.9)	20.5 ± 1.2 (19.6 – 22.2)
Lower tooth row length (IM3I)	17.2 ± 0.6 (16.4 – 17.9)	16.9 ± 0.6 (16.2 – 17.9)	15.9 ± 0.4 (15.5 – 16.6)	16.9 ± 0.4 (16.5 – 17.6)	14.6 ± 1.4 (12.8 – 16.0)
Coronoid process (CP)	9.7 ± 0.4 (9.2 – 10.3)	9.3 ± 0.3 (8.9 – 10.0)	8.3 ± 0.5 (7.8 – 8.9)	9.3 ± 0.3 (8.9 – 9.9)	7.1 ± 0.8 (6.2 – 8.2)
Length of angular process (LAP)	9.4 ± 0.3 (8.8 – 9.9)	8.8 ± 0.3 (8.1 – 9.1)	8.2 ± 0.5 (7.6 – 8.9)	8.7 ± 0.7 (8.1 – 9.7)	6.7 ± 0.9 (6.0 – 7.8)

PHYLOGENETIC ANALYSES

Seven new partial mitochondrial *cyt b* sequences (1136 bp) of *Hylomys* from Genting Highlands, Peninsular Malaysia were obtained and have been uploaded in the GenBank under accession number MW023076 – MW023082. Another 21 *Hylomys cyt b* sequences with one sequence of *Otohylomys megalotis*, four sequences of *Neohylomys hainanensis* and two sequences of *Neotetracus sinensis* were retrieved from the GenBank records (Appendix B). Two *cyt b* sequences of *Erinaceus europaeus* and *Erinaceus roumanicus*

were used as outgroups (Appendix B). These sequences were aligned and edited using the Applied Biosystems Sequence Scanner software version 1.0. Pairwise genetic distances among all the *cyt b* gene fragments were estimated using Kimura's two-parameter (K2P) model (Kimura 1980) which was calculated using the MEGA X software (Kumar et al. 2018). We constructed the phylogenetic trees inferred with maximum likelihood (ML) and Bayesian analysis (BA) methods to illustrate the relationships of all sampled taxa. ML trees were constructed using 1000 bootstrap pseudoreplicates in

PAUP version 4.0a (Felsenstein 1985) to estimate the bootstrap support (BS) to the tree nodes. BA trees were constructed using the best-fit-model, GTR+I+G which was determined using the Akaike Information Criterion (AIC) (Akaike 1974) using jModelTest V.2.1.7 (Darriba et al. 2012). The BA trees parameters achieved stationarity after a burn-in period of about 1 million generations by sampling every 1000 generations implementing Metro-Coupled Markov Chain Monte Carlo (MCMCMC). This was performed in MrBayes version 3.0 (Huelsenbeck & Ronquist 2001). The tree was then viewed and edited using the TreeView version 1.6.6 software. The Bayesian posterior probabilities (BPP) were estimated for each node and stated in the tree.

RESULTS

Seven individuals of the *Hylomys* samples were trapped in the mossy forest of Genting Highlands, Pahang with coordinates of 3° 26'16.1" N 101° 47' 6.5" E (Figure 1, the elevation of 1000 – 1700 m above sea level). The external morphology measurements are given in Table 1. The *Hylomys* samples from Genting Highlands have an average of 133.4 mm of head to body measurements and weigh between 43 and 80 g. The average of the hind foot length is approximately 25.9 mm. The tail is short and hairless, measuring between 8 and 16 mm and the ear length is between 14 and 17 mm. The adult *Hylomys* male has dark brown fur on the upper part while the under part is covered with light yellow-brown fur (Figure 2). External measurements based on comparisons were taken for a few available *Hylomys* species (Table 1). The dwarf gymnure, *H. parvus* (Sumatra) has the smallest head to body length with an average of 108.5 mm while *H. dorsalis* (Borneo) recorded the largest head to body length with an average of 135.9 mm. In addition, *H. parvus* has the longest tail with an average length of 23.1 mm while *H. dorsalis* has the largest ear size (25.0 mm). The average length of hind foot for all species is almost the same and ranges between 20.8 and 25.9 mm. The weight between species and subspecies cannot be compared due to the unavailable dataset.

Skull measurements were compared among the *Hylomys* species from Peninsular Malaysia and the Sunda Islands (Table 2). Among species, the overall average skull size of *H. parvus* (Sumatra) is smaller compared to that of *H. maxi* (Peninsular Malaysia), *H. suillus* (Java) and *H. dorsalis* (Borneo) except for the average length of upper molariform (PM3). *Hylomys maxi* (Peninsular Malaysia) recorded 10 average skull measurements (GLS, ROB, PPL, PPD, IM3S, P4M3,

MAL, IM3I, CP and LAP) larger than *H. suillus* (Java) and *H. dorsalis* (Borneo). In contrast, the Javan species, *H. suillus* has nine smaller average skull measurements (GLS, CBL, ROL, PPL, IM3S, MAL, IM3I, CP and LAP) compared to *H. maxi* and *H. dorsalis*. However, two average skull measurements (M3B and PM3) of *H. suillus* are larger than the other two. Nine out of sixteen craniodental measurements (GLS, CBL, BB, ROL, ROB, PPD, M3B, PM3 and P4M3) of the *Hylomys* samples from Genting Highlands are related to the other *H. maxi* samples from Peninsular Malaysia. The comparison between samples cannot be done among localities within Peninsular Malaysia because the details on the sampling localities (i.e., Cameron Highlands, Fraser's Hills) of the museum specimens are unavailable.

We conducted a PCA (Figure 3) on 16 skull variables of *Hylomys* samples from Genting Highlands, along with four other taxa of *Hylomys* from the museum specimens which are *H. maxi* (Peninsular Malaysia), *H. suillus* (Java), *H. dorsalis* (Borneo) and *H. parvus* (Sumatra). The first two principal components (PC1 and PC2) displayed 75.7% of the total variation (Appendix C). PC1 which accounts for 66.1% of the total variance has a low positive correlation below 0.3 with all the factors studied, indicating that this factor is correlated with the general skull size. On the other hand, PC2 which accounts for 9.6% of the total variation has the highest positive correlation (0.269) with P4M3 and the highest negative correlation (-0.699) with IOB. PC2 has low correlations with the other factors expressing broadness of the skull. The PCA plot in Figure 3 shows that the samples from Genting Highlands and *H. maxi* from other localities in Peninsular Malaysia overlap with each other. On the other hand, *H. parvus* from Sumatra forms a distinct cluster on the left of the first axis, indicating that this species is smaller in skull size compared to the other taxa, *H. maxi*, *H. suillus* and *H. dorsalis*. *Hylomys maxi* from Peninsular Malaysia along with *Hylomys* samples from Genting Highlands have positive loadings on factor 1 and factor 2, indicating that the species tend to be larger in size but specifically narrower in skull size when compared to *H. suillus* from Java and *H. dorsalis* from Borneo.

Phylogenetic trees inferred from *cyt b* (1336 bp) with 609 conserved sites, 527 variable sites and 500 parsimony-informative sites highly support monophyly in *Hylomys* samples from Genting Highlands, Peninsular Malaysia with the species, *H. maxi* from Cameron Highlands and Fraser's Hill, Peninsular Malaysia as well as Sumatra, Indonesia with BPP of 1.0

TABLE 3. Percent pairwise corrected (K2P) average genetic distance between groups among genus *Hylomys* and three other species in different genera, *Neotetracus sinensis*, *Neohylomys hainanensis*, and *Otomyomys megalotis* inferred from mitochondrial *cyt b* sequences. Their geographical distributions are stated in parenthesis. *Erinaceus europaeus* and *Erinaceus roumanicus* were included as outgroups

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 <i>Hylomys</i> sp. (Genting Highlands, P. Malaysia)	-														
2 <i>H. maxi</i> (Sumatra)	1.0	-													
3 <i>H. maxi</i> (Fraser's Hill, P. Malaysia)	3.0	3.0	-												
4 <i>H. maxi</i> (Cameron Highlands, P. Malaysia)	6.0	6.0	4.0	-											
5 <i>H. suillus</i> (Java, Indonesia)	18.0	17.0	17.0	17.0	-										
6 <i>H. parvus</i> (Sumatra, Indonesia)	18.0	19.0	19.0	19.0	13.0	-									
7 <i>H. dorsalis</i> (Sabah, Malaysia Borneo)	19.0	19.0	20.0	20.0	17.0	7.0	-								
8 putative sp. 2 (Vietnam)	19.0	19.0	20.0	20.0	22.0	20.0	20.0	-							
9 <i>H. microtinus</i> (Vietnam)	20.0	20.0	21.0	21.0	18.0	19.0	19.0	20.0	-						
10 <i>H. siamensis</i> (Thailand)	20.0	20.0	21.0	21.0	18.0	20.0	19.0	19.0	6.0	-					
11 putative sp. 1 (Yunnan, China)	21.0	21.0	21.0	21.0	16.0	15.0	16.0	18.0	9.0	10.0	-				
12 <i>N. hainanensis</i> (Hainan, China)	23.0	23.0	24.0	24.0	24.0	21.0	23.0	24.0	25.0	27.0	24.0	-			
13 <i>N. sinensis</i> (Sichuan and Yunnan, China)	26.0	26.0	27.0	27.0	27.0	28.0	28.0	27.0	28.0	28.0	29.0	22.0	-		
14 <i>O. megalotis</i> (Laos)	30.0	30.0	32.0	32.0	28.0	28.0	29.0	29.0	30.0	30.0	30.0	31.0	31.0	-	
15 Outgroups	32.0	32.0	34.0	34.0	32.0	34.0	34.0	34.0	35.0	34.0	38.0	34.0	36.0	35.0	-

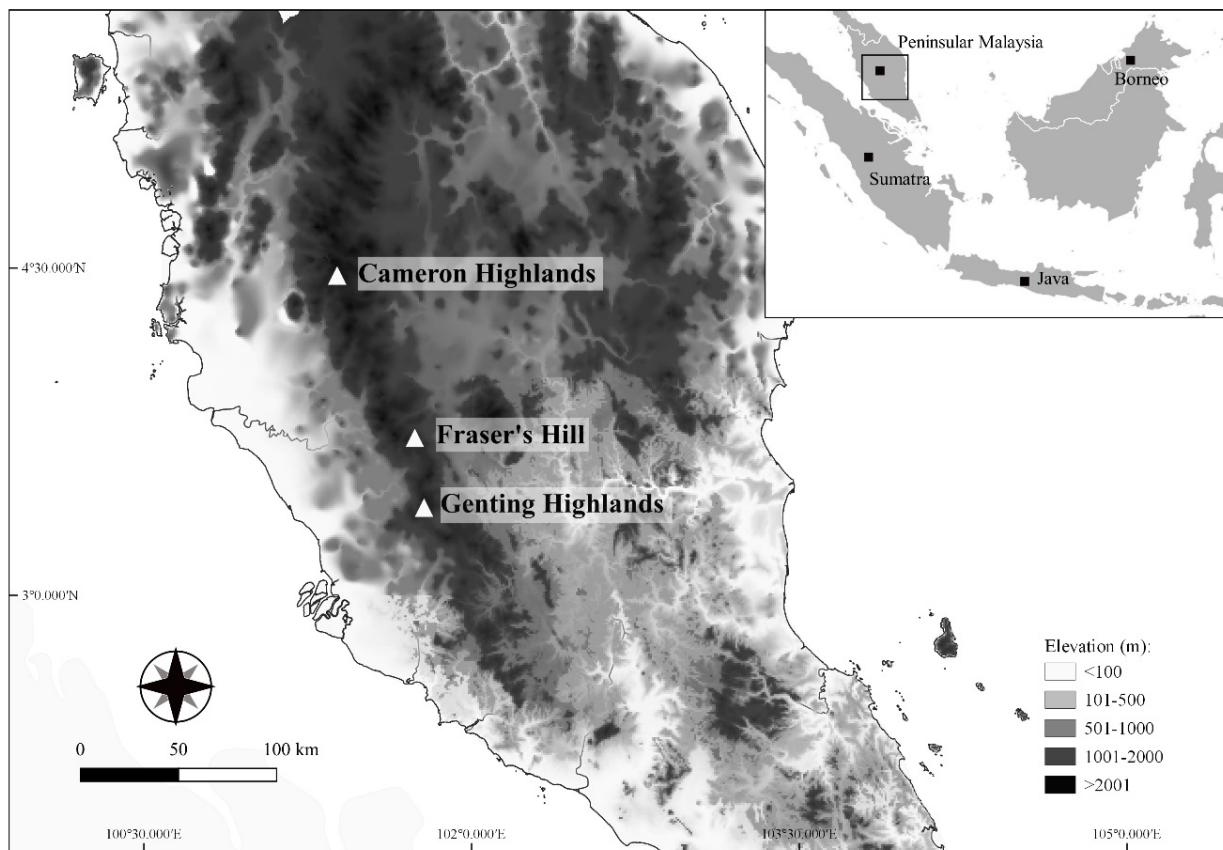


FIGURE 1. The map showing the sampling localities for this study, Genting Highlands, Pahang with two others previous highland localities for the isolated *Hylomys* populations, Fraser's Hill and Cameron Highlands with elevation more than 1000 m above sea level published by Ruedi et al. (1994)



FIGURE 2. The image of a live adult *Hylomys* male captured from Genting Highlands, Pahang (elevation of 1000 - 1700 metres above sea-level) using cage traps (Photo courtesy from Hasmahzaiti Omar)

and BS of 100% (Figure 4). Within these *H. maxi* samples, the genetic distance between the samples from Genting Highlands and Cameron Highlands as well as those from Sumatra and Cameron Highlands is the highest with K2P=6.0, whereas the genetic distance between the samples from Genting Highlands and Fraser's Hill as well as Sumatra and Fraser's Hill, both recorded at K2P=3.0 (Table 3). Interestingly, the genetic distance of the samples from Genting Highlands and Sumatra is the

lowest with K2P=1.0. In addition, the genetic distance between Fraser's Hill and Cameron Highlands recorded K2P=4.0. These results and the geographic location, as well as morphological analyses, strongly support that the *Hylomys* samples from Genting Highlands represent *H. maxi*. *Hylomys maxi* formed a monophyletic cluster with *H. dorsalis* from Sabah in Malaysia Borneo, *H. suillus* from Java and *H. parvus* from Sumatra separating them from the Indochinese species.

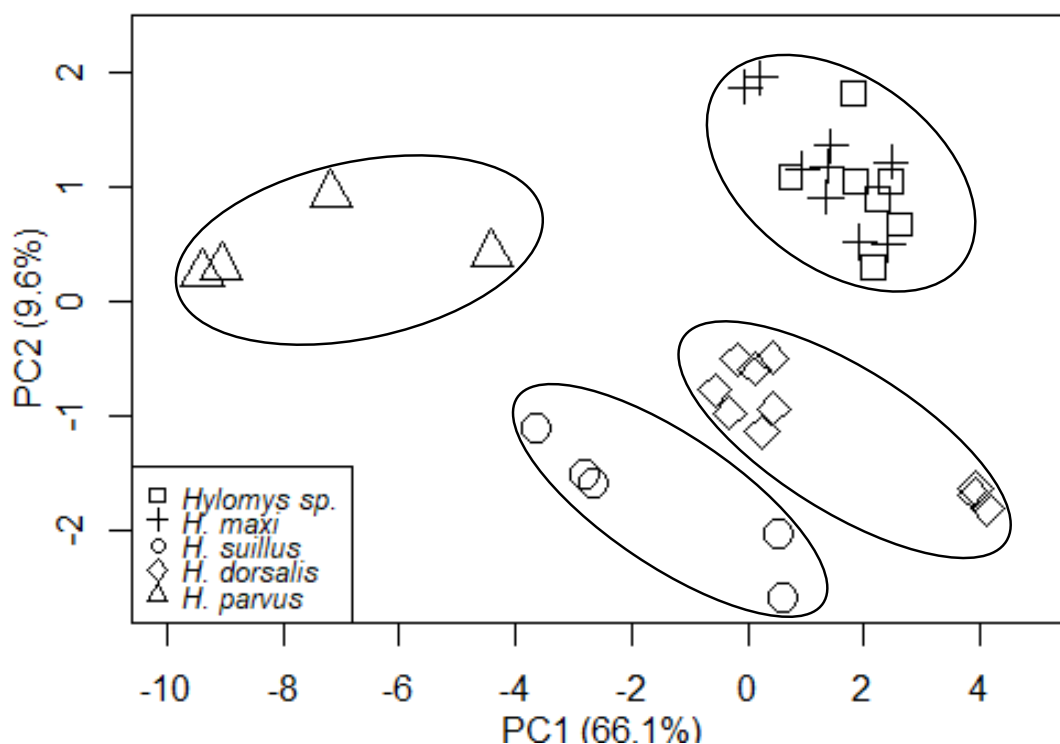


FIGURE 3. Projection of the first two principal components of PCA based on 16 skull measurements of seven *Hylomys* samples (*Hylomys* sp.) from Genting Highlands with the other four taxa: *H. maxi* (Peninsular Malaysia), *H. suillus* (Java), *H. dorsalis* (Borneo) and *H. parvus* (Sumatra). The taxa labelled according to each shape was given in the box

On the other hand, the Indochinese samples which are *H. microtinus* and *Hylomys* sp. from Yunnan, China formed a sister clade with a genetic distance of K2P>20.0 and K2P>21.0 while *Hylomys* from Bu Gia Map and Vat Tien, Vietnam located at ancestral position with a genetic distance of K2P>19.0. In clades from Peninsular Malaysia and Sunda, *H. maxi*, *H. dorsalis*, *H. suillus* and *H. parvus* formed separate clades with strong support values. However, among these clades, *H. maxi*

showed high genetic distance percentages with other *Hylomys* species (K2P>18.0%). The *Hylomys* lineage is also well-separated from the other two genera of the tribe Hylomyini, *Neohylomys* and *Neotetracus* species supported with high statistical value (BPP=1.0/BS=100) as well as the monotypic species of tribe Otohylomyini, *Otohylomys megalotis*. The outgroup species, *Erinaceus europaeus* and *Erinaceus roumanicus* were also split

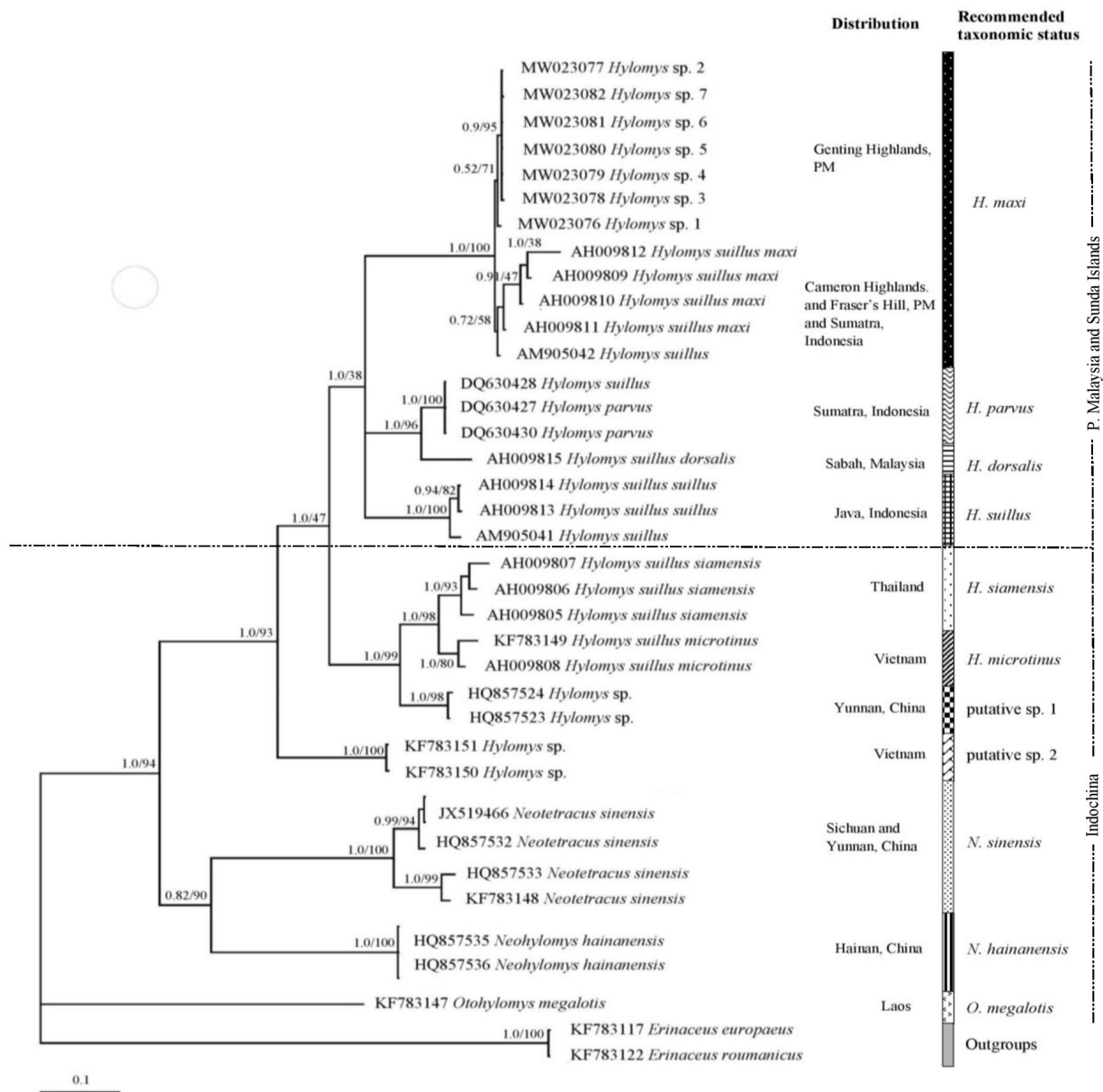


FIGURE 4. Bayesian consensus tree of phylogenetic relationship for genus *Hylomys* with three other species in different genera, *Neotetracus sinensis*, *Neohylomys hainanensis*, and *Otohylomys megalotis* inferred from the cytochrome *b* (1136 bp) using *Erinaceus* sp. as outgroups. Their distribution and recommended taxonomic status are illustrated. Nodal support is represented by Bayesian posterior probabilities (BPP) and ML bootstrap support (BS)

from others in the trees with a high genetic distance of $K2P > 23.0\%$ and $K2P > 32.0\%$, respectively.

DISCUSSION

The extensive distribution of the *Hylomys* species across

several islands of Sundaland to the Indochina regions (Corbet & Hill 1992; Nor 2001; Ruedi & Fumagalli 1996; Ruedi et al. 1994; Smith et al. 2008) raised questions whether this single taxon migrated to these regions during the past epoch or due to inadequate taxonomic

assessment. From a taxonomic perspective, the external and skull measurement comparisons could not be conducted among the Peninsular Malaysia localities and between Peninsular Malaysia samples with *H. maxi* from Sumatra due to unavailable samples. However, the present study shows similarities between the *Hylomys* species from Genting Highlands with other *H. maxi* samples from Peninsular Malaysia via univariate (descriptive statistics) and multivariate analysis (PCA). The differences in external morphologies and skull variations between these two species (*H. maxi* and *H. suillus*) strengthen the species divergence where *H. maxi* from Peninsular Malaysia tends to be larger in body size with shorter tail and has larger but narrower skull size compared to *H. suillus* from Java. The differences in external and skull measurement of *Hylomys* samples from Genting Highlands with other taxa, *H. dorsalis* and *H. parvus* also can be noticed where *H. parvus* has the smaller overall sizes among them.

According to Avise et al. (1987), the mitochondrial DNA can usually reflect phylogenetic and phylogeographic histories well at both intra- and inter-specific levels due to rapid evolutionary rate. Thus, we reconstruct the phylogenetic tree of the *Hylomys* lineage based on the *cyt b* genetic sequences to perceive their genetic relationships. Our phylogenetic analyses support the monophyletic clade of *Hylomys* species from Peninsular Malaysia and Sunda Islands (*H. maxi*, *H. suillus*, *H. dorsalis*, and *H. parvus*) which separated from the Indochina clade (*H. siamensis*, *H. microtinus* and two putative species, Figure 4) with genetic divergence of $K2P > 19\%$. The analysis was based on a single maternal gene. Therefore, these results should be reconsidered and re-examined by involving multiple loci. Our tree supported the separation of these two major clades which coincide with the phylogenetic tree of He et al. (2012). Both populations probably separated due to the formation of Isthmus of Kra which acted as a geographic barrier.

The newly caught *Hylomys* samples from Genting Highlands (*Hylomys* sp.) formed a strongly supported clade with the *H. maxi* samples from Cameron Highlands and Fraser's Hill, Peninsular Malaysia and Sumatra, Indonesia. Genetic distances however showed a closer relationship between *Hylomys* from Genting Highlands and Sumatra ($K2P=1.0$) compared to the *Hylomys* samples which were collected from a distance less than 200 km to the north of Peninsular Malaysia (Fraser's Hill: $K2P=3.0$ and Cameron Highlands: $K2P=6.0$). This suggests a lack of gene flow among these isolated populations. Geographically, all three highland localities

(Genting Highlands, Fraser's Hill and Cameron Highlands) are located in Pahang on the Titiwangsa range also known as the Central Belt (Khoo & Tan 1983). The isolation of the population might have occurred during the high sea-level of interglacial periods (Bird et al. 2005; Esselstyn et al. 2009; Hewitt 2000) where the ancestral *Hylomys* persisted in refuges on these highlands with further dispersal and vicariant events in the Mesozoic epoch where the mountain ranges in Peninsular Malaysia were uplifted and folded through orogenesis (Cavendish 2007). The theory by Ruedi and Fumagalli (1996) considered *H. maxi* to be a native of the Malay Peninsula which invaded Sumatra only recently. This might explain the notably low intra-specific genetic distance between *H. maxi*.

On the other hand, *H. maxi* from Peninsular Malaysia and Sumatra as well as *H. suillus* from Java, Indonesia (Arnason et al. 2008) appeared to be separated into different clades in the phylogenetic tree (Figure 4) and are highly genetically diverged ($K2P=18\%$, Table 3). This relatively high genetic distance supports the potential species-level divergence (Bradley & Baker 2001) and we confirm that the *Hylomys* samples from Genting Highlands can be classified as a valid species, *Hylomys maxi*. The possible mechanism of this speciation occurs probably due to vicariant scenarios, sea level fluctuations, savannah barriers to forest-dependent organisms (Esselstyn et al. 2009; Gorog et al. 2004; Heaney et al. 2005). For instance, the palaeoecological data of the presence of continuous lowland forests between Sumatra, Malay Peninsula and Borneo except for Java when the Sunda Shelf was exposed during glacial maxima (Raes et al. 2014). This could possibly be the reason for the Javan *Hylomys* for not reaching other Sundaic regions.

Two sympatric species from Sumatra, *H. parvus* and *H. maxi* are well-differentiated from the phylogenetic tree. *Hylomys parvus* was found to be closely related to *H. dorsalis* from Borneo with high statistical support. This result coincides with the findings of Ruedi et al. (1994), who investigated the phylogenetic relationships of *Hylomys* based on *H. parvus* and other *H. suillus* samples. Previously, Ruedi et al. (1994) proposed that the *Hylomys* samples from Borneo are given a subspecies status under the monophyletic clade of *Hylomys suillus* while *H. parvus* as a distinct species due to its phylogenetic position derived from Nei's unbiased genetic distances. Nevertheless, the present study shows that *H. parvus* is within the *H. suillus* clade specifically related to *H. dorsalis* (Figure 4) with a relatively high genetic divergence between them ($K2P=7.0$) indicating that this

Bornean gymnure is a valid species. Allopatric speciation might occur between the Sumatran species, *H. parvus* and among other *Hylomys* species from Peninsular Malaysia and Sunda Islands (*H. maxi*, *H. suillus*, and *H. dorsalis*) where the range-shift of populations might have occurred during the Pliocene-Pleistocene epoch when the sea-level changed and the land bridges formed might have affected the *Hylomys* lineages in the Sunda Shelf (Bird et al. 2005; Hewitt 2000).

CONCLUSIONS

In accordance with observed morphological variation, genetic diversification, and geographical isolation, we confirm that the *Hylomys* samples collected from Genting Highlands and other localities from Peninsular Malaysia along with the Sumatran samples as a single valid species, *Hylomys maxi*. Apart from the sharing of knowledge and new genetic and morphological data from Genting Highlands samples for future references, this study also clarified the existence of two *Hylomys* lineages which are the i) Sundaic lineage which consists of *Hylomys suillus* Müller, 1840 – Java, Indonesia, *Hylomys maxi* Sody, 1933 – Peninsular Malaysia and Sumatra, Indonesia, *Hylomys dorsalis* Thomas, 1888 – Borneo (Sabah, Malaysia) and *H. parvus* – Robinson and Kloss, 1916 Sumatra and ii) Indochinese lineage which consists of *H. siamensis* Kloss, 1916 – Thailand, *H. microtinus* Thomas, 1925 – Vietnam, putative sp. 1 – Yunnan, China and putative sp. 2 – Vietnam. Both latter putative *Hylomys* species require further assessment for species identification and classification for a better understanding of the *Hylomys* lineages.

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APPENDIX A. List of *Hylomys* skull specimens examined from Museum of Zoology, Universiti Malaya (MZUM) and Lee Kong Chian Natural History Museum, Singapore (ZRC) with number of specimen (n), museum specimen numbers and localities

Species (no. of specimen)	Museum specimen no.	Localities
Museum of Zoology, Universiti Malaya (MZUM)		
<i>Hylomys</i> sp. (n=7)	MZUM(M)-S898-904	Genting Highlands, Malaysia
<i>H. s. maxi</i> (n=8)	MZUM(M)-S133(a)	Peninsular Malaysia
	MZUM(M)-S135(18)	Pahang, Malaysia
	MZUM(M)-S135(72)	-
	MZUM(M)-S135(130)	Peninsular Malaysia
	MZUM(M)-S135(303)	Pahang, Malaysia
	MZUM(M)-S135(308),	Pahang, Malaysia
	MZUM(M)-S136(17)	Pahang, Malaysia
	MZUM(M)-S136(305)	Pahang, Malaysia
Lee Kong Chian Natural History Museum, Singapore (ZRC)		
<i>H. s. maxi</i> (n=1)	ZRC.4.5048	Selangor, Malaysia
<i>H. s. suillus</i> (n=5)	ZRC.4.5042	Banjoewangi, Java, Indonesia

	ZRC.4.5037	Banjoewangi, Java, Indonesia
	ZRC.4.5040	Banjoewangi, Java, Indonesia
	ZRC.4.5038	Banjoewangi, Java, Indonesia
	ZRC.4.5039	Banjoewangi, Java, Indonesia
<i>H. s. dorsalis</i> (n=10)	ZRC.4.5020	Mt. Kinabalu, Sabah, Malaysia
	ZRC.4.5017	Mt. Kinabalu, Sabah, Malaysia
	ZRC.4.5022	Mt. Kinabalu, Sabah, Malaysia
	ZRC.4.5016	Mt. Kinabalu, Sabah, Malaysia
	ZRC.4.5012	Mt. Kinabalu, Sabah, Malaysia
	ZRC.4.5013	Mt. Kinabalu, Sabah, Malaysia
	ZRC.4.5019	Mt. Kinabalu, Sabah, Malaysia
	ZRC.4.5023	Mt. Kinabalu, Sabah, Malaysia
	ZRC.4.5018	Mt. Kinabalu, Sabah, Malaysia
	ZRC.4.5014	Mt. Kinabalu, Sabah, Malaysia
	ZRC.4.5034	Dran, S. Annam, Thailand
<i>H. parvus</i> (n=4)	ZRC.4.3434	Korinchi Peak, Sumatra
	ZRC.4.3430	Korinchi Peak, Sumatra
	ZRC.4.5030	Korinchi Peak, Sumatra
	ZRC.4.5045	Korinchi Peak, Sumatra

APPENDIX B. List of accession numbers and references for mitochondrial *cyt b* sequences of the *Hylomys*, *Neohylomys*, *Neotetracus*, and *Otohylomys* with two outgroup species; *Erinaceus europaeus* and *Erinaceus roumanicus* retrieved from GenBank for phylogenetic analyses

Species	GenBank accession number	Localities	References
Ingroup			
<i>Hylomys maxi</i>	MW023076	Genting Highlands, Pahang, Malaysia	In this study
	MW023077	Genting Highlands, Pahang, Malaysia	In this study
	MW023078	Genting Highlands, Pahang, Malaysia	In this study
	MW023079	Genting Highlands, Pahang, Malaysia	In this study
	MW023080	Genting Highlands, Pahang, Malaysia	In this study
	MW023081	Genting Highlands, Pahang, Malaysia	In this study
	MW023082	Genting Highlands, Pahang, Malaysia	In this study

<i>Hylomys</i> sp.	HQ857524	Yunnan, China	He et al. (2011)
	HQ857523	Yunnan, China	He et al. (2011)
	DQ630428	-	Dubey et al. (2007)
	AM905042	Peninsular Malaysia	Arnason et al. (2008)
	AM905041	Java, Indonesia	Arnason et al. (2008)
	KF783151	Bu Gia Map, Vietnam	Bannikova et al. (2014)
	KF783150	Cat Tien, Vietnam	Bannikova et al. (2014)
<i>H. s. maxi</i>	AH009812	Cameron Highlands, Pahang, Malaysia	Ruedi & Fumagalli (1996)
	AH009811	Sumatra, Indonesia	Ruedi & Fumagalli (1996)
	AH009810	Fraser's Hill, Pahang, Malaysia	Ruedi & Fumagalli (1996)
	AH009809	Fraser's Hill, Pahang, Malaysia	Ruedi & Fumagalli (1996)
<i>H. s. suillus</i>	AH009814	Java, Indonesia	Ruedi & Fumagalli (1996)
	AH009813	Java, Indonesia	Ruedi & Fumagalli (1996)
<i>H. s. dorsalis</i>	AH009815	Sabah, Malaysia	Ruedi & Fumagalli (1996)
<i>H. s. microtinus</i>	KF783149	Son La, Vietnam	Bannikova et al. (2014)
	AH009808	Hoa Binh, Vietnam	Ruedi & Fumagalli (1996)
<i>H. s. siamensis</i>	AH009807	Chaiyaphum, Thailand	Ruedi & Fumagalli (1996)
	AH009806	Chaiyaphum, Thailand	Ruedi & Fumagalli (1996)
<i>H. parvus</i>	DQ630430	Sumatra, Indonesia	Dubey et al. (2007)
	DQ630427	Sumatra, Indonesia	Dubey et al. (2007)
Ingroup			
<i>Neohylomys hainanensis</i>	HQ857535	Hainan, China	He et al. (2011)
	HQ857536	Hainan, China	He et al. (2011)
<i>Neotetracus sinensis</i>	HQ857533	Yunnan, China	He et al. (2011)
	HQ857532	Yunnan, China	He et al. (2011)
	KF783148	Yunnan, China	Bannikova et al. (2014)
	JX519466	Sichuan, China	Lu et al. (2012)
<i>Otohylomys megalotis</i>	KF783147	Ban Doy, Laos	Bannikova et al. (2014)
Outgroup			
<i>Erinaceus europaeus</i>	KF783117	Moscow, Russia	Bannikova et al. (2014)
<i>Erinaceus roumanicus</i>	KF783122	Moscow, Russia	Bannikova et al. (2014)

APPENDIX C. Factor loadings for the principal components analysis (PCA) based on 16 skull characters of *Hylomys* species. Eigenvalues and percentage of total variance for both factors are also included

Skull Characters	Factor 1	Factor 2
Greatest length of skull (GLS)	0.294	0.110
Condylobasal length (CBL)	0.227	<0.001
Braincase breadth (BB)	0.234	-0.362
Interorbital breadth (IOB)	<0.001	-0.699
Rostral length (ROL)	0.268	<0.001
Rostral breadth (ROB)	0.251	<0.001
Postpalatal length (PPL)	0.275	0.213
Postpalatal depth (PPD)	0.271	<0.001
Breadth at third molars (M3B)	0.220	-0.393
Length of upper molariform (PM3)	<0.001	-0.193
Length of upper tooth row (IM3S)	0.293	<0.001
P4 to M3 length (P4M3)	0.249	0.269
Mandibular length (MAL)	0.295	<0.001
Lower tooth row length (IM3I)	0.275	0.102
Coronoid process (CP)	0.270	<0.001
Length of angular process (LAP)	0.285	<0.001
Eigenvalues	10.6	1.5
% Total variance	66.1	9.6