Sains Malaysiana 55(5)(2026): 774-784
http://doi.org/10.17576/jsm-2026-5505-01

Phylogenetic Relationships of House Shrews with the Genetic Divergence from Peninsular Malaysia

(Hubungan Filogenetik Cencurut Rumah dengan Perbezaan Genetik dari Semenanjung Malaysia)

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Received: 17 August 2024/Accepted: 24 April 2026

ABSTRACT

The house shrew (*Suncus murinus*) displays a wide distribution extending from the coastal region of eastern Africa and reaching southern East Asia through West Asia, South Asia, and Southeast Asia. Here, we used mitochondrial cytochrome *b* sequence data to investigate the phylogenetics relationships of *S. murinus* in Peninsular Malaysia. Our findings indicated that the populations from southwestern and eastern Peninsular Malaysia exhibit minimal genetic divergence, with K2P values between inter-populations and intra-populations for both populations ranging from 0.00% to 0.03%. Based on phylogenetic trees, these populations were grouped together in one monophyletic clade with those shrews from southern Myanmar, Java, Vietnam, Japan, Taiwan and *S. m. caerulescens*. However, these two populations were genetically distinct from the population in northwestern Peninsular Malaysia. This northwestern population was unique as it formed a single, strongly supported monophyletic clade, with the genetic distances between the northwestern and southwestern Peninsular Malaysia populations being high (K2P > 5%). Aside from that, we also found a major phylogeographic split between the northwestern and southwestern Peninsular Malaysia populations. This biogeographic boundary is located around 4°N the coast of Lumut, Perak. The observed genetic divergence indicates historical isolation and independent evolution among house shrew populations in Peninsular Malaysia, underscoring the need for further research to understand the contributing factors to these findings.

Keywords: Cytochrome *b*; genetic relationships; Malaysia; *Suncus murinus*

ABSTRAK

Cencurut rumah (*Suncus murinus*) mempunyai taburan yang luas merangkumi kawasan pantai timur Afrika sehingga selatan Asia Timur melalui Asia Barat, Asia Selatan dan Asia Tenggara. Di sini, kami menggunakan data jujukan sitokrom *b* mitokondria untuk mengkaji hubungan filogenetik *S. murinus* di Semenanjung Malaysia. Hasil penemuan kami menunjukkan bahawa populasi dari selatan barat dan timur Semenanjung Malaysia menunjukkan perbezaan genetik yang minimum, dengan nilai K2P antara populasi dan intra-populasi untuk kedua-dua populasi adalah antara 0.00% hingga 0.03%. Berdasarkan pohon filogenetik, populasi ini dikelompokkan bersama dalam satu klad monofiletik dengan cencurut rumah dari selatan Myanmar, Pulau Jawa, Vietnam, Jepun, Taiwan dan *S. m. caerulescens*. Bagaimanapun, kedua-dua populasi ini berbeza secara genetik dengan populasi di utara barat Semenanjung Malaysia. Populasi utara barat laut ini unik kerana ia membentuk satu klad monofiletik yang disokong kuat dengan jarak genetik antara populasi utara barat dan selatan barat Semenanjung Malaysia adalah tinggi (K2P > 5%). Selain itu, kami juga menemukan perpecahan filogeografi antara populasi utara barat dan selatan barat Semenanjung Malaysia. Sempadan biogeografi ini terletak di sekitar 4°U pantai Lumut, Perak. Perbezaan genetik yang diperhatikan menunjukkan terdapat pengasingan sejarah dan evolusi bebas dalam kalangan populasi cencurut rumah di Semenanjung Malaysia menekankan keperluan untuk penyelidikan lanjut untuk memahami faktor penyumbang kepada hasil penemuan ini.

Kata kunci: Hubungan genetik; Malaysia; sitokrom *b*; *Suncus murinus*

INTRODUCTION

Suncus murinus or house shrew (Soricidae: Crocidurinae) is often associated with human dwellings such as houses and inhabits the edge of forests (Burney et al. 2004; Heideman et al. 1987; Kitchener, Schmitt & Maharadatunkamsi 1994; Rana & Prakash 1979; Ruedi et al. 1996). The current distribution of this terrestrial animal is prominently influenced by human movements either over land or maritime routes (Advani & Rana 1981; Seymour et al. 2005). The species are broadly distributed across East Africa, South Asia, the Middle East, Southeast Asia, and East Asia (Chen et al. 2016; Hutterer 2005; Motokawa 2015). Phillips (1980) described four subspecies of *S. murinus* across Sri Lanka and India: *S. m. murinus*, *S. m. kandianus*, *S. m. caerulescens,* and *S. montanus*. However, *S. montanus* is regarded as an independent species by several taxonomists due to the blackish color of its fur, significantly smaller in body size and preference for forests (Corbet & Hill 1992, 1991). Meegaskumbura, Meegaskumbura and Schneider (2010) also proposed that *S. montanus* as a distinct species based on molecular and morphological data. However, Kikuchi et al. (2020) and Ohdachi et al. (2017, 2016) treated *S. montanus* as a species complex due to the incomplete investigation of the genetic relationships within these two species. Therefore, this study considered *S. murinus* and *S. montanus* as a single taxon, the *Suncus murinus-S. montanus* species complex, similar approach to Kikuchi et al. (2020) and Ohdachi et al. (2017, 2016).

At early investigations, Yamagata et al. (1990) found lack of mitochondrial DNA (mtDNA) variation among Japanese and Indonesian shrews using RFLP techniques. Meanwhile, Ruedi et al. (1996) showed very low levels of genetic differentiation within the *S. murinus* lineage in Southeast Asia and Japan compared to Indian populations. Yamagata et al. (1995) later identified three distinct mtDNA haplotype groups: continental, island and Malay. Kurachi et al. (2007a) classified shrews into three major groups based on mtDNA: South Asia, Southeast Asia and the Malay group, while Kurachi et al. (2007b) found higher genetic divergence within Southeast Asia compared to South Asia, contradicting earlier findings based on allozyme data. Subsequent work by Ohdachi et al. (2016) using cytochrome *b* identified several differentiated clades among shrews from Pakistan, Sri Lanka and Myanmar. Additionally, Ohdachi et al. (2017) found that shrews from Tanzania and Iran shared a mitochondrial cytochrome *b* haplotype and proposed connections between shrews from Yemen and Madagascar/Comoros. Besides, their study also found a closer relationship between Réunion Island shrews and those from Malaysia and Myanmar.

In Peninsular Malaysia, there is a notable lack of recent phylogeographic data of house shrew populations which are representing a significant gap in understanding their regional and global phylogenetic contexts. Addressing this gap is essential for exploring the evolutionary dynamics of house shrews in the region and developing effective conservation strategies amidst environmental changes and habitat fragmentation. This study aims to fill this knowledge gap by providing new insights through the phylogenetic analysis using mitochondrial cytochrome *b* gene among house shrew populations in Peninsular Malaysia. It represents the first comprehensive effort to analyse both phylogenetic and morphometric aspects of house shrew populations across the region.

MATERIALS AND METHODS

SAMPLE COLLECTION AND ETHICAL APPROVAL

The house shrew samples were collected from numerous sites from Peninsular Malaysia which comprise Ulu Gombak (Selangor), Lumut (Perak), Bukit Katil (Melaka), Alor Setar (Kedah), Port Dickson (Negeri Sembilan), Kuala Terengganu (Terengganu), Wang Kelian (Perlis), Ayer Itam (Pulau Pinang), Kuantan (Pahang), Jerantut (Pahang) and Kota Bharu (Kelantan) (Figure 1). A total of 100 live traps with dimensions of 45 cm × 16 cm × 16 cm containing fried chicken bait were set up surrounding at human settlements such as house yards, shops, drainage areas, and gardens. This study was conducted for seven successive nights.

All live or dead shrews were recorded for their gender and subsequently euthanized using carbon dioxide inside modified containers. The skin and skulls were extracted from the bodies and the remaining body residues were preserved as wet specimens at the Museum of Zoology, Institute of Biological Sciences, Faculty of Science, Universiti Malaya for future studies. For molecular study, the liver tissue was extracted and then stored at -80° C freezer. All samples collected were approved by The Department of Wildlife and National Parks Peninsular Malaysia with a permit reference number; JPHL&TN(IP): 100-34/1.24 Jld. 7(6). Meanwhile, the protocol of animal handling was approved by Universiti Malaya Institutional Animal Care and Use Committee (IACUC) with protocol number ISB/10/06/2016/NHMI (R).

DNA EXTRACTION

By using GF-1 Tissue DNA Extraction Kit provided by Vivantis Technologies, the tissue samples of house shrews were conducted. For DNA amplifications of cytochrome b gene, the PCR master mix with a total volume of 25.0 µL consisted of 8.0 µL of DNA (30 ng/µL), 2.0 µL of primers (15 mM), 2.5 µL of dNTP (10 mM), 2.0 µL of MgCl2 (25 mM), 2.5 µL of GoTaq ® Flexi buffer and 0.2 µL of Go Taq DNA polymerase (6.0 u/µL). One primer pair, MVZ 05 and MVZ 14 were used for this procedure (Smith & Patton 1999) and the condition was set up 40 cycles of denaturation at 94 °C for 35 s, annealing temperature at 46 °C for 45 s, extension at 72 °C for one minute, accordingly. The innuPREP PCRpure Kit (Analytik Jena) was used to purify the PCR products. Lastly, the PCR products were sequenced at First Base Co. (Selangor, Malaysia).

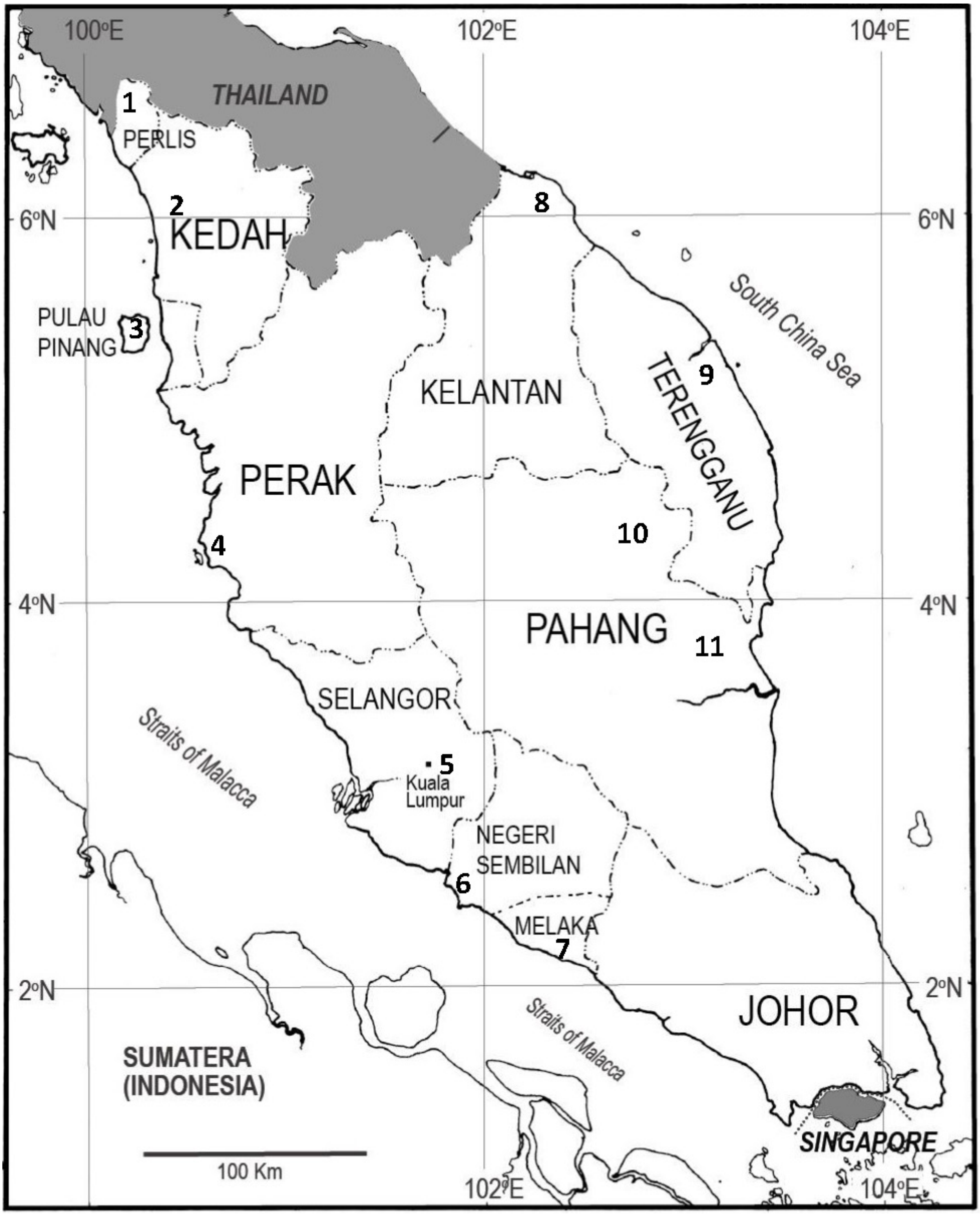

FIGURE 1. Sample collections sites of house shrews in Peninsular Malaysia. West Peninsular Malaysia: 1. Wang Kelian, Perlis, 2. Alor Setar, Kedah, 3. Ayer Itam, Pulau Pinang and 4. Lumut, Perak. 5. Ulu Gombak, Selangor, 6. Port Dickson, Negeri Sembilan and 7. Bukit Katil, Melaka. East Peninsular Malaysia: 8. Kota Bharu, Kelantan, 9. Kuala Terengganu, Terengganu, 10. Kuantan, Pahang and 11. Jerantut, Pahang

SEQUENCE ALIGNMENT AND PHYLOGENETIC RECONSTRUCTION

The editing and alignment procedures using Clustal W (Thompson, Higgins & Gibson 1994) of all sequences for the house shrews were conducted using the MEGA 11.0 software (Tamura, Stecher & Kumar 2021). The pairwise distances of the cytochrome *b* gene were estimated using the Kimura two-parameter (K2P) model (Kimura 1980) to get insight into the genetic divergence among groups. The pairwise distances of the cytochrome *b* gene were estimated using the Kimura two-parameter (K2P) model (Kimura 1980) to get insight into the genetic divergence among clades and localities in Peninsular Malaysia. For the pairwise distances of localities in Peninsular Malaysia, the localities in Perak were divided into two groups according to clades: Perak 1 (PRMY1, PRMY3, PRMY4 and PRMY6) and Perak 2 (PRMY2 and PRMY5). A total of 75 sequences including one outgroup was used to construct the phylogenetic inference inferred from cytochrome *b* gene. One sequence of *Suncus etruscus* was obtained from GenBank as an outgroup.

The construction of phylogenetic trees was performed using the Bayesian Inference (BI) and Maximum Likelihood (ML) analysis. For BI method, the best-fit model which is GTR + I + G was selected using Model test version 3.7 (Posada & Crandall 1998). To generate BA tree, MrBayes 3.0 (Huelsenbeck & Ronquist 2001) was utilised with one million generations using Metropolis-Coupled Markov Chain Monte Carlo (MCMCMC) variant with trees sampled every 1000 generations. The tree reached stationarity after a burn period of 230,000 generations. The Tamura-Nei model was used to build the ML tree with 1000 bootstrap replicates to acquire the best-fitting model evolution of DNA sequence. ML tree was constructed using the MEGA 11.0 software (Tamura, Stecher & Kumar 2021). The values above the branches correspond to bootstrap support for ML (BS_{ML}) and posterior probabilities for BI (BI) with 1000 replicates. For each node, the confidence support is illustrated as BS_{ML}/BI.

MORPHOLOGICAL STUDIES

Five external morphology traits and 14 skull characteristics were assessed, following the methodologies outlined by Omar et al. (2013) and Ruedi (1995) (Figure 2). Descriptive statistics for the external body and skull measurements were calculated (Tables 3 & 4). However, the limited sample size constrained our ability to conduct a comprehensive morphological diagnosis and detailed morphometric analysis.

RESULTS

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES

A total of 74 sequences of house shrews were generated in the region of cytochrome *b* (data not shown). The final sequence alignment had lengths of 1140 nucleotide bases for this gene. The average base composition in cytochrome b was as follows: T=32.3%, C=25.4%, A=29.0% and G=13.3%. Among the aligned sites, 269 sites (23.6%) were variable, with 143 sites being parsimony-informative. The phylogenetic trees of BI and ML analysis methods of cytochrome *b* sequences showed the similar tree topology; therefore, only BI topology was displayed in Figure 3. The phylogenetic analysis of the cytochrome *b* data in this study suggest that house shrews can be sorted into seven major lineages; (1) a mixture of lineages from southwestern and eastern Peninsular Malaysia, Vietnam, Java, southern Myanmar, Taiwan, China, Japan and *S. m. caerulescens*, (2) *S. m. murinus* lineages, (3) lineages from Shan state, Myanmar, (4) lineages from Chin state, Myanmar, (5) shrews from northwestern Peninsular Malaysia, (6) lineages from Nay Pyi Taw, Myanmar and (7) *S. m. kandianus*-*S. montanus* complex (Figure 3).

In this study, the populations from Peninsular Malaysia were divided into two clades. The southwestern and eastern Peninsular Malaysia populations were grouped together in a monophyletic clade with *S. m. caerulescens* and other accessions from southern Myanmar, Java, Vietnam, Taiwan, China and Japan (BS_{ML}= 98%, BI =1.00). Meanwhile, the shrews from northwestern Peninsular Malaysia formed a single monophyletic clade with a strong bootstrap support (BS_{ML}= 99%, BI = 1.00). The populations from Perak were divided into two clades. Some shrews from Perak clustered together within a large clade consisting of shrews from southwestern and eastern Peninsular Malaysia, as well as other accessions from southern Myanmar, Java, Vietnam, Taiwan, China, and Japan. The other shrews from Perak were grouped together within a distinct monophyletic clade with shrews from northwestern Peninsular Malaysia.

Within cytochrome *b* sequences, the intraspecies K2P genetic distance between clades spanned from 1.33% to 7.56% (Table 1). The highest genetic distance, reaching 7.56%, was noted between Myanmar 2 and *S. m. kandianus*-*S. montanus* complex. The lowest genetic distance was observed between the clade consisting of *S. m. murinus* lineages and the clades consisting of *S. m. caerulescens* and other shrews from major localities in Asia (K2P = 1.33%). Among localities in Peninsular Malaysia, the highest genetic distance was found between Penang and Melaka (K2P = 5.86%) (Table 2). The genetic distances between inter-populations and intra-populations in southern and eastern Peninsular Malaysia are low, ranging from 0.00% to 0.03%. For localities in northwestern Peninsular Malaysia, the genetic distances between each other were also low (K2P = 0.00-0.03%). However, the genetic distances between northwestern Peninsular Malaysia and southwestern Peninsular Malaysia were high (K2P > 5%). Overall, most genetic distances between mtDNA clades or populations in Peninsular Malaysia revealed more than 5% genetic divergence.

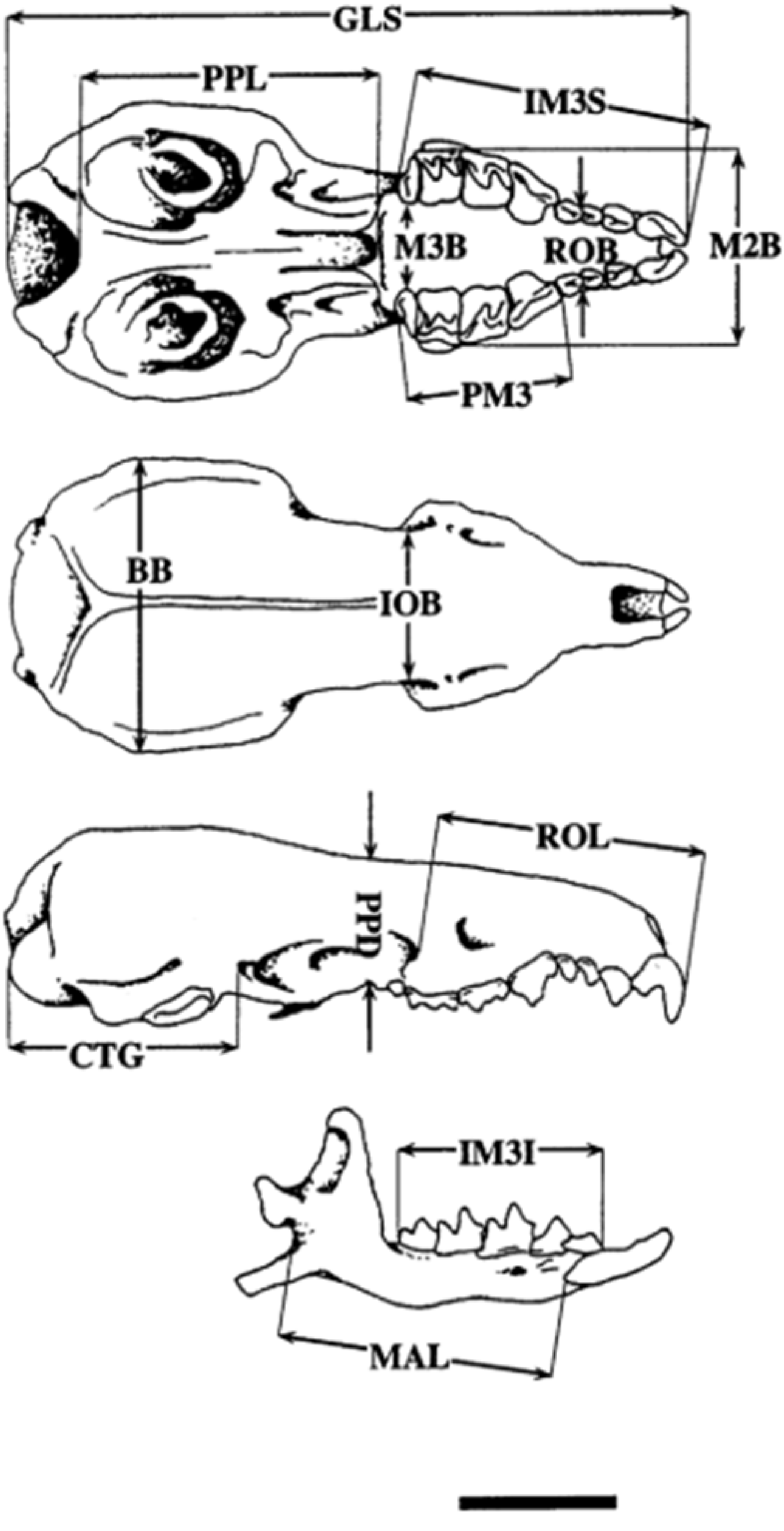

FIGURE 2. Illustrations of 14 skull measurements taken following protocol from Heaney and Timm (1983) and Ruedi (1995). The black bar corresponds to 5 mm (Photo sourced from Ruedi 1995)

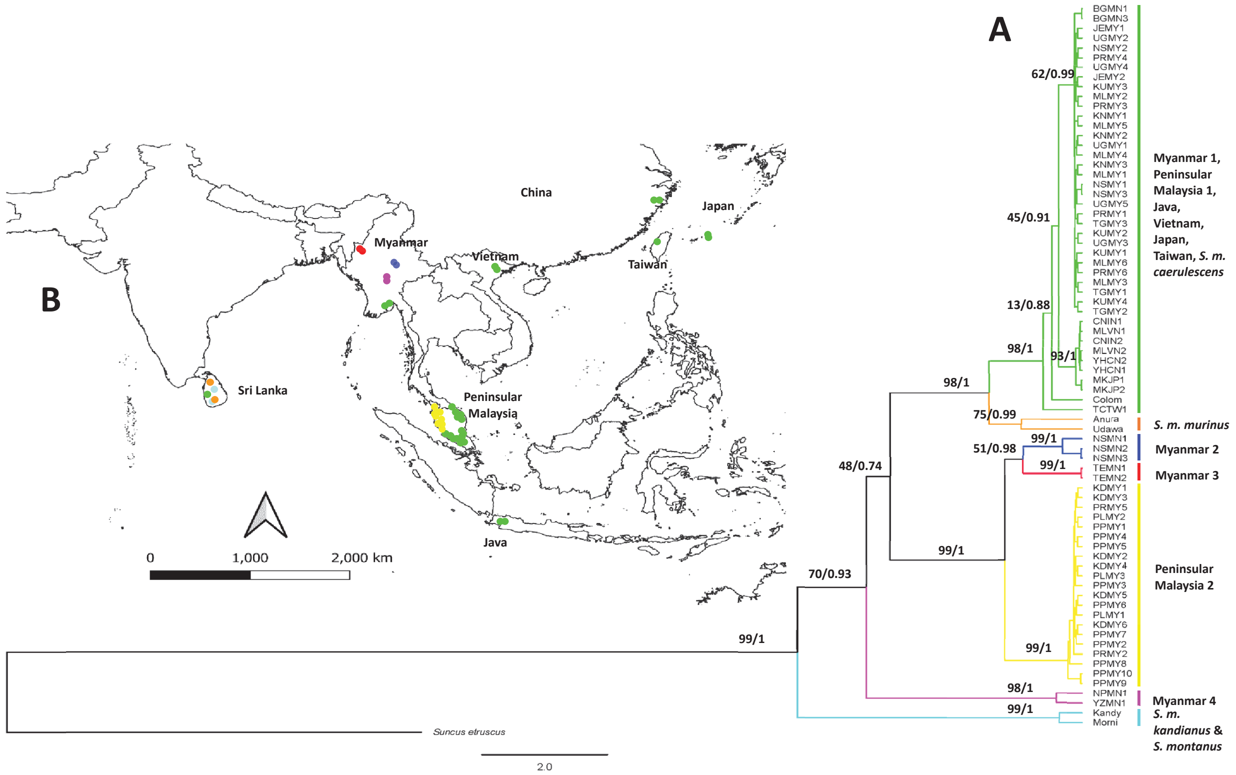

FIGURE 3. A) Phylogenetic tree derived from ML and BI constructed based on the cytochrome *b* genetic sequences. Values above the branches correspond to bootstrap support (BS) and posterior probabilities (BI) with 1000 replicates. For each node, the confidence support is illustrated as BS_{ML}/BI. (B) Geographic map showing population background used in the study

TABLE 1. Genetic distance (%) of house shrews between mtDNA clades based on the Kimura-2-Parameter model inferred from cytochrome *b* gene

	MN 1, PM 1, TH, JV, VN, JP, TW, *S. m. caerulescens*	*S. m. murinus*	Myanmar 2	Myanmar 3	PM 2	Myanmar 4	*S. m. kandianus-S. montanus*
MN 1, PM 1, TH, JV, VN, JP, TW, *S. m. caerulescens*	-						
S. m. murinus	1.33	-					
Myanmar 2	5.00	4.50	-				
Myanmar 3	5.51	5.15	1.74	-			
PM 2	5.71	4.55	1.75	2.64	-		
Myanmar 4	5.16	4.02	5.11	6.48	5.38	-	
S. m. kandianus-S. montanus	6.67	5.75	7.56	7.38	7.28	5.92	-

MN, Myanmar; PM, Peninsular Malaysia; TH, Thailand; JV, Java; VN, Vietnam; JP, Japan; TW, Taiwan

TABLE 2. Genetic distance (%) of house shrews between localities in Peninsular Malaysia based on the Kimura-2-Parameter model inferred from cytochrome *b* gene

		Perlis	Kedah	Penang	Perak 1	Perak 2	Selangor	Negeri Sembilan	Melaka	Pahang	Terengganu	Kelantan
NORTH PM	Perlis	-										
	Kedah	0.00	-									
	Penang	0.03	0.03	-								
	Perak 1	0.01	0.01		-							
SOUTH PM	Perak 2	5.75	5.75	5.78	5.61	-						
	Selangor	5.70	5.70	5.73	5.58	0.00	-					
	Negeri Sembilan	5.73	5.74	5.77	5.60	0.00	0.00	-				
	Melaka	5.85	5.85	5.86	5.69	0.03	0.03	0.03	-			
EAST PM	Pahang	5.75	5.75	5.78	5.61	0.00	0.00	0.00	0.03	-		
	Terengganu	5.75	5.75	5.78	5.61	0.00	0.00	0.00	0.03	0.00	-	
	Kelantan	5.75	5.75	5.78	5.61	0.00	0.00	0.00	0.03	0.00	0.00	-

PM, Peninsular Malaysia

MORPHOLOGICAL ANALYSES

The external measurements for the house shrews across different Peninsular Malaysia regions highlight distinct variations. Shrews from the Southwest region recorded the highest head to body length (109.7 mm), while those in the Northwest were the shortest (106.4 mm). Similarly, tail lengths and hindfoot lengths showed minor variations, with Southwest populations exhibiting slightly longer measures in both dimensions. Weight differed marginally, with Eastern populations being heavier (92.9 g) compared to the Northwestern shrews (86.4 g). The 14 cranio-dental metrics across regional populations showed that Southwest specimens had generally larger cranial measurements. This was especially notable in the greatest skull length (GLS), where the Southwest shrews had a mean GLS of 31.0 mm compared to 29.0 mm in the Northwest population. Similar patterns were observed for other cranio-dental traits, suggesting possible geographic influences on morphological characteristics.

DISCUSSION

The mitochondrial cytochrome *b* gene is previously utilized in systematic studies to resolve divergences at multiple taxonomic levels, including those of house shrews (Kikuchi et al. 2020; Meegaskumbura, Meegaskumbura & Schneider 2010; Ohdachi et al. 2017, 2016). In this study, the cytochrome *b* gene has been used as a prominent marker to investigate the intraspecific variation among house shrews in Asia with focus on Peninsular Malaysia. Based on base composition of cytochrome *b* in this study, the thymine (T) and adenine (A) are more abundant than cytosine (C) and guanine (G). This pattern reflects the AT-rich nature of mitochondrial genomes, which is a common characteristic that has been documented in previous studies on mammalian mtDNA (Andrews et al. 1999).

Based on mtDNA phylogenetic trees, shrews from southwestern and eastern Peninsular Malaysia, Vietnam, Java, southern Myanmar, Taiwan, China, Japan, and *S. m. caerulescens* grouped together in a clade. This suggests there might be migration of shrews between all these areas. This clade is consistent with the findings of Kikuchi et al. (2020) and Ohdachi et al. (2016), with the addition of samples from southwestern and eastern Peninsular Malaysia in this study. Within this clade, the shrews from Java, Vietnam, Japan, and China formed a strongly supported sub-clade (BS_{ML} = 93%, BI = 1.00). This data indicates close genetic relationships between these populations. This data is consistent with the findings of Kurachi et al. (2007a) and Yamagata et al. (1995, 1990), who reported no mtDNA variation among shrews from Japan, Vietnam, and Indonesia. It also aligns with Ohdachi et al. (2016), who suggested that house shrews in Japan (Okinawa), southern China, and Indonesia (Java) likely migrated with human assistance, although the direction of migration remains unclear. Furthermore, Ohdachi et al. (2017) proposed that shrews in Java, China, and Japan probably migrated from Vietnam based on median-joining haplotype network data.

In addition, we have made a novel discovery of two distinct populations in Peninsular Malaysia. Shrew lineages from southwestern and eastern Peninsular Malaysia are part of a single monophyletic clade that includes shrews from East Asia, Java, Vietnam, southern Myanmar and *S. m. caerulescens*. In northwestern Peninsular Malaysia, we discovered another unique lineage of shrews, as

TABLE 3. External measurements (in mm) of 53 captured individuals from this study based on populations including the measured characters, mean, minimum to maximum and standard deviation (std. dev.)

Characters	Northwest (n=20)			East (n=13)			Southwest (n=20)		
	Mean	Minimum-maximum	Std. dev.	Mean	Minimum-maximum	Std. dev.	Mean	Minimum-maximum	Std. dev.
Head and body length (HB)	106.4	100-113	2.54	108.3	101-116	2.75	109.7	104-117	2.29
Tail (T)	68.2	67-70	1.48	68.7	67-71	1.51	69.4	67-72	1.67
Ear length (EL)	11.5	11.2-12.2	0.82	11.6	11.3-12.4	0.76	12.1	11.7-12.5	0.64
Hindfoot (HF)	18.6	18.0-19.2	0.75	19.3	18.9-19.7	0.73	19.2	18.3-20.1	0.77
Weight (g)	86.4	53-119	23.6	92.9	63-121	24.8	90.7	52-128	26.5

TABLE 4. Mean and standard deviation of 14 cranio-dental measurement (in mm) for 53 specimens collected in Peninsular Malaysia with the range of values (minimum-maximum) given in parenthesis. Sample size (n) is given for each population

Skull measurements	Peninsular Malaysia		
	Northwest (n=20)	East (n=13)	Southwest (n=20)
Greatest length skull (GLS)	29.0 ± 1.4 (26.1-30.4)	29.6 ± 0.5 (28.8-30.3)	31.0 ± 0.2 (30.9-31.3)
Length of upper tooth row (IM3S)	12.7± 0.4 (11.8-13.2)	12.8± 0.1 (12.7-13.1)	13.5± 0.2 (13.3-13.7)
Post-palatal length (PPL)	12.6± 0.8 (10.9-13.5)	12.6± 0.4 (12.0-13.1)	13.5± 0.3 (13.1-13.7)
Length of upper molariform (PM3)	6.8 ± 0.2 (6.6-7.1)	7.0 ± 0.1 (6.8-7.1)	7.4 ± 0.3 (7.2-7.7)
Rostral breadth (ROB)	3.5 ± 0.1 (3.4-3.6)	3.6 ± 0.1 (3.4-3.8)	3.7 ± 0.2 (3.5-3.9)
Breadth at third molars (M3B)	3.3 ± 0.1 (3.2-3.6)	3.4 ± 0.2 (3.1-3.6)	3.3 (3.3-3.4)
Breadth at second molars (M2B)	8.7 ± 0.3 (8.3-9.0)	8.6 ± 0.2 (8.3-8.8)	9.1 ± 0.1 (9.0-9.2)
Braincase breadth (BB)	12.1 ± 0.6 (10.9-12.7)	12.0 ± 0.4 (11.4-12.6)	12.5 ± 0.1 (12.3-12.5)
Interorbital breadth (IOB)	5.5 ± 0.2 (5.1-5.7)	5.7 ± 0.2 (5.5-5.9)	5.7 ± 0.1 (5.7-5.8)
Rostral length (ROL)	11.5 ± 0.4 (10.7-11.9)	11.6 ± 0.2 (11.3-11.9)	12.3 (12.3-12.4)
Post- palatal depth (PPD)	5.3 ± 0.3 (4.9-5.6)	5.4 ± 0.2 (5.1-5.7)	5.6 ± 0.1 (5.4-5.7)
Condyle to glenoid length (CTG)	10.5± 0.8 (8.8-11.2)	10.8± 0.4 (10.0-11.2)	11.7± 0.2 (11.4-11.9)
Lower tooth row length excluding first incisor (IM3I)	8.5 ± 0.2 (8.2-8.8)	8.4 ± 0.7 (7.2-8.8)	8.9 ± 0.2 (8.7-9.1)
Mandibular length (MAL)	11.9 ± 0.5 (10.8-12.5)	12.0 ± 0.2 (11.8-12.2)	12.1 ± 1.0 (11.5-14.3)

this population formed a single and strongly supported monophyletic clade. Thus, this study suggests the possible formation of an endemic haplotype in northwestern Peninsular Malaysia. Interestingly, a new finding in the present study showed a major phylogeographic split between the northwestern and southwestern Peninsular Malaysia populations. This biogeographic boundary is located around 4°N the coast of Lumut, Perak. Lumut is located at coastal zone whereby climatic conditions are extremely variable (Ranasinghe 2016). Similarly, Kubiak et al. (2017) reported that two cases of sympatry have been recognized for Subterranean rodents of the genus Ctenomys in the coastal dunes of southern Argentina and southern Brazil. Therefore, this study suggested Lumut as the contact area between both populations.

Based on Bradley and Baker (2001), DNA sequence divergence values could serve as an additional data source for defining an appropriate 'measure of taxonomic rank'. Based on mtDNA clades, the genetic distance between the western Myanmar populations (Myanmar 2) and the *S. m. kandianus-S. montanus* complex was the highest (K2P > 7%), indicating significant genetic divergence between these two groups. Conversely, the lowest genetic distance between *S. m. murinus* and other shrews in southern Myanmar, southwestern and eastern Peninsular Malaysia and East Asia including *S. m. caerulescens* had been observed, suggests ongoing or recent gene flow between these populations.

The genetic distances among shrew populations in Peninsular Malaysia show interesting patterns of genetic variation and potential barriers to gene flow. The genetic distances between inter-populations and intra-populations in southern and eastern Peninsular Malaysia are notably low, ranging from 0.00% to 0.03%. Similarly, the genetic distances between localities in northwestern Peninsular Malaysia are also low (K2P = 0.00-0.03%). These low genetic distances indicate high genetic similarity and implies that the populations are either not geographically isolated or that barriers to gene flow are minimal, allowing for genetic exchange and maintaining homogeneity. However, a contrast pattern is observed when comparing the genetic distances between northwestern and southwestern Peninsular Malaysia, with values exceeding 5% (K2P > 5%). Among the localities studied, the highest genetic distance was observed between Penang and Melaka, with a K2P value of 5.86%. These high genetic distances indicate substantial genetic divergence suggesting that these regions have been isolated from each other, likely due to geographical or ecological barriers that restrict gene flow. The significant genetic differentiation implies that the northwestern and southwestern populations have evolved independently, accumulating genetic differences over time.

The findings from this study showed that most genetic distances between mtDNA clades or populations in Peninsular Malaysia showed more than 5% genetic divergence. Bradley and Baker (2001) suggested that a cytochrome *b* sequence divergence greater than 2% could indicate species-level divergence, while Avise (2000) stated that intraspecific divergences are rarely greater than 2% and most are less than 1%. This significant divergence implies that some of these populations might represent distinct species or subspecies.

For morphological studies, the variations in both external and cranio-dental morphology observed among house shrew populations in Peninsular Malaysia likely reflect regional adaptation influenced by environmental and genetic factors. Previous studies have highlighted similar trends among small mammals where population-specific traits are shaped by the habitat and ecological demands (Cardini 2020). The observed size differences, particularly the higher head to body length in the Southwest population may align with findings in other shrew studies where environmental conditions like climate impact body size. For instance, Shoma, Feroz and Hasan (2016) documented comparable body size variations in house shrews across Japan, Sri Lanka, and Bangladesh, attributing the differences to environmental pressures that influence body dimensions. The larger cranial features in the Southwest population may suggest dietary or ecological adaptations unique to that region as larger skulls and teeth are often linked to different feeding strategies. Studies on other small mammals have shown that cranial morphologies can vary due to feeding and environmental adaptations (Noftz & Calede 2022).

CONCLUSION

In conclusion, our study confirmed significant genetic divergence between house shrew populations in Peninsular Malaysia. This divergence suggests historical isolation and independent evolution in certain regions. These findings highlight the complex population dynamics of house shrews in this area. On the other hand, the morphological differences highlighted in this study suggest a regional adaptation of house shrews in Peninsular Malaysia. Further research incorporating larger samples and geometric morphometrics would improve our understanding of the underlying causes of these variations.

ACKNOWLEDGEMENTS

A special gratitude to the Japan Society for the Promotion of Science (JSPS) Core-to-Core Program (JSPS KAKENHI Grant JP18H03602) and the Universiti Malaya research grant (BKS031-2017) for supplying financial assistance for this present study. We would also like to express gratitude to all team members and staffs of the Faculty of Science, Universiti Malaya cooperated in this study. We declare that there is no conflict of interest. Supplementary data is available upon requests from the corresponding author.

REFERENCES

Advani, R. & Rana, B.D. 1981. Food of the house shrew, *Suncus murinus sindensis* in the Indian desert. *Acta Theriologica* 26: 133-134.

Andrews, R.M., Kubacka, I., Chinnery, P.F., Lightowlers, R.N., Turnbull, D.M. & Howell, N. 1999. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nature Genetics* 23(2): 147.

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

Bradley, R.D. & Baker, R.J. 2001. A test of the genetic species concept: Cytochrome-b sequences and mammals. *Journal of Mammalogy* 82(4): 960-973.

Burney, D.A., Burney, L.P., Godfrey, L.R., Jungers, W.L., Goodman, S.M., Wright, H.T. & Timothy Jull, A.J. 2004. A chronology for late prehistoric Madagascar. *Journal of Human Evolution* 47(1-2): 25-63.

Chen, S., Wei, H., Peng, H. & Yong, B. 2016. The complete mitogenome of Asian House Shrews, *Suncus murinus* (Soricidae). *Mitochondrial DNA* 27(2): 1127-1128.

Cardini, A. 2020. Modern morphometrics and the study of population differences: Good data behind clever analyses and cool pictures? *The Anatomical Record* 303(11): 2747-2765.

Corbet, G.B. & Hill, J.E. 1992. *The Mammals of the Indomalayan Region: A Systematic Review*. Oxford: Oxford University Press.

Corbet, G.B. & Hill, J.E. 1991. *A World List of Mammalian Species*. Oxford: Oxford University Press.

Heaney, L.R. & Timm, R. 1983. *Relationships of Pocket Gophers of the Genus Geomys from the Central and Northern Great Plains.* Museum of National History, University of Kansas.

Heideman, P., Heaney, L., Thomas, R. & Erickson, K. 1987. Patterns of faunal diversity and species abundance of non-volant small mammals on Negros Island, Philippines. *Journal of Mammalogy* 68(4): 884-888.

Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755. doi:10.1093/bioinformatics/17.8.754

Hutterer, R. 2005. Order Soricomorpha. In *Mammals Species of the World: A Taxonomical and Geographical Reference*, 3rd ed., edited by Wilson, D.E. & Reeder, D.A. Maryland: Johns Hopkins University Press.

Kikuchi, F., Aoki, K., Ohdachi, S.D., Tsuchiya, K., Motokawa, M., Jogahara, T., Sơn, N.T., Bawm, S., Lin, K.S., Thwe, T.L., Gamage, C.D., Ranorosoa, M.C., Omar, H., Maryanto, I., Suzuki, H., Tanaka-Taya, K., Morikawa, S., Mizutani, T., Suzuki, M., Yanagihara, R. & Arai, S. 2020. Genetic diversity and phylogeography of *Thottapalayam thottimvirus* (*Hantaviridae*) in Asian house shrew (*Suncus murinus*) in Eurasia. *Frontiers in Cellular and Infection Microbiology* 10: 438. doi: 10.3389/fcimb.2020.00438

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16(2): 111-120.

Kitchener, D., Schmitt, L. & Maharadatunkamsi. 1994. Morphological and genetic variation in *Suncus murinus* (Soricidae: Crocidurinae) from Java, Lesser Sunda islands, Maluku and Sulawesi, Indonesia. *Mammalia* 58(3): 433-452.

Kubiak, B.B., Gutiérrez, E.E., Galiano, D., Maestri, R. & de Freitas, T.R.O. 2017. Can niche modeling and geometric morphometrics document competitive exclusion in a pair of subterranean rodents (Genus *Ctenomys*) with tiny parapatric distributions? *Scientific Reports* 7(1): 16283.

Kurachi, M., Chau, B.L., Dang, V.B., Dorji, T., Yamamoto, Y., Nyunt, M.M., Maeda, Y., Chhum-Phith, L., Namikawa, T. & Yamagata, T. 2007a. Population structure of wild musk shrews (*Suncus murinus*) in Asia based on mitochondrial DNA variation, with research in Cambodia and Bhutan. *Biochemical Genetics* 45(3-4): 165-183.

Kurachi, M., Kawamoto, Y., Tsubota, Y., Chau, B.L., Dang, V.B., Dorji, T., Yamamoto, Y., Nyunt, M.M., Maeda, Y., Chhum-Phith, L., Namikawa, T. & Yamagata, T. 2007b. Phylogeography of wild musk shrew (*Suncus murinus*) populations in Asia based on blood protein/enzyme variation. *Biochemical Genetics* 45(7-8): 543-563.

Meegaskumbura, S., Meegaskumbura, M. & Schneider, C.J. 2010. Systematic relationships and taxonomy of *Suncus montanus* and *S. murinus* from Sri Lanka. *Molecular Phylogenetics and Evolution* 55(2): 473-487.

Motokawa. 2015. *Suncus murinus* (Linnaeus, 1766). In *The Wild Mammals of Japan*, 2nd ed., edited by Ohdachi, S.D., Ishibashi, Y., Iwasa, M.A., Fukui, D. & Saitoh, T. Kyoto: Shoukadoh.

Noftz, L.A. & Calede, J.J. 2022. Multivariate analyses of skull morphology inform the taxonomy and evolution of geomyoid rodents. *Current Zoology* 69(4): 456-474.

Ohdachi, S.D., Kinoshita, G., Nasher, A.K., Yonezawa, T., Arai, S., Kikuchi, F., Linn, K. & Bawm, S. 2017. Re-evaluation of the phylogeny based on mitochondrial cytochrome *b* gene in the house shrew, *Suncus murinus-S. montanus* species complex, with special reference to Yemen and Myanmar populations. *Journal of Wildlife Diseases* 1: 79-87.

Ohdachi, S.D., Kinoshita, G., Oda, S., Motokawa, M., Jogahara, T., Arai, S., Nguyen, S.T., Suzuki, H., Katakura, K., Bawm, S., Min, M.Z., Thwe, T.L., Gamage, C.D., Hashim, R., Omar, H., Maryanto, I., Ghadirian, T., Ranorosoa, M.C., Moribe, J. & Tsuchiya, K. 2016. Intraspecific phylogeny of the house shrews, *Suncus murinus-S. montanus* species complex based on the mitochondrial cytochrome *b* gene. *Mammal Study* 41(4): 229-238.

Omar, H., Hashim, R., Bhassu, S. & Ruedi, M. 2013. Morphological and genetic relationships of the *Crocidura monticola* species complex (Soricidae: Crocidurinae) in Sundaland. *Mammalian Biology* 78(6): 446-454.

Phillips, W.W.A. 1980. *A Manual of the Mammals of Sri Lanka*. Colombo: Wildlife and Nature Protection Society of Sri Lanka.

Posada, D. & Crandall, K.A. 1998. MODELTEST: Testing the model of DNA substitution. *Bioinformatics* 14(9): 817-818.

Rana, B.D. & Prakash, I. 1979. Reproductive biology and population structure of the house shrew, *Suncus murinus sindensis*, in Western Rajasthan. *Zeitschrift für Säugetierkunde* 44: 333-343.

Ranasinghe, R. 2016. Assessing climate change impacts on open sandy coasts: A review. *Earth-Science Reviews* 160: 320-332.

Ruedi, M. 1995. Taxonomic revision of shrews of the genus *Crocidura* from the Sunda Shelf and Sulawesi with description of two new species (Mammalia: Soricidae). *Zoological Journal of the Linnean Society* 115(3): 211-265.

Ruedi, M., Courvoisier, C., Vogel, P. & Catzeflis, F.M. 1996. Genetic differenciation and zoogeography of Asian *Suncus murinus* (Mammalia:Soricidae). *The Biological Journal of the Linnean Society* 57: 307-316.

Seymour, A., Varnham, K., Roy, S., Harris, S., Bhageerutty, L., Church, S., Harris, A., Jennings, N.V., Jones, C., Khadun, A., Mauremootoo, J., Newman, T., Tatayah, V., Webbon, C. & Wilson, G. 2005. Mechanisms underlying the failure of an attempt to eradicate the invasive Asian musk shrew *Suncus murinus* from an island nature reserve. *Biological Conservation* 125(1): 23-35.

Shoma, S.F., Feroz, M.M. & Hasan, M.K. 2016. Morphometry of Asian house shrew (*Suncus murinus*) in Jahangirnagar University Campus. *Bangladesh Journal of Zoology* 43(2): 313-319.

Smith, M.F. & Patton, J.L. 1999. Phylogenetic relationships and the radiation of sigmodontine rodents in South America: Evidence from cytochrome *b*. *Journal of Mammalian Evolution* 6: 89-128.

Tamura, K., Stecher, G. & Kumar, S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology Evolution* (38): 3022-3027.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22(22): 4673-4680.

Yamagata, T., Ohishi, K., Faruque, M.O., Masangkay, J.S., Ba-Loc, C., Vu-Binh, D., Mansjoer, S.S., Ikeda, H. & Namikawa, T. 1995. Genetic variation and geographic distribution on the mitochondrial DNA in local populations of the musk shrew, *Suncus murinus*. *Genes & Genetic Systems* 70(3): 321-337.

Yamagata, T., Tanaka, Y., Ishikawa, A., Namikawa & Tomita, T. 1990. Genetic relationship among the musk shrews, *Suncus murinus* insectivora, inhabiting islands and the continent based on mitochondrial DNA types. *Biochemical Genetics* 28(3-4): 185-195.

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