

Phylogeography of Red Spiny Rats *Maxomys surifer* (Miller, 1900) (Mammalia: Rodentia: Muridae) based on Cytochrome *b* in peninsular Thailand

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ABSTRACT. – Spiny rats (Genus *Maxomys*) are an endemic rodent species in Southeast Asia belonging to the family Murinae. There are currently 18 species recognized in the genus *Maxomys*, with a general distribution extending from mainland Southeast Asia eastward over the Sunda Shelf to some nearby islands. The phylogeography of *M. surifer* (Miller, 1900) in Thailand, particularly in peninsular Thailand, is presented in this study. A phylogenetic tree and haplotype networks were reconstructed to comprehend the geographic variation in the study area. Analyses included 898 base pairs of mitochondrial cytochrome *b* sequence data for 92 individuals of *M. surifer*, comprising 59 newly obtained sequences and 33 sequences from GenBank. The phylogenetic tree with the maximum likelihood inference revealed five highly divergent lineages within *M. surifer*. Three lineages (Clades I - III) were found in peninsular Thailand: Clade I includes specimens from a small area in the southern part of the peninsula; Clade II consists of specimens from both the mainland of peninsular Thailand and nearby islands in the Andaman Sea, including those from the type locality; Clade III comprises specimens from the central to northern parts of peninsular Thailand, extending toward the Kra Isthmus. Clades IV and V involved specimens from the Indochinese subregion. The results extend a view proposed in previous studies that *M. surifer* comprises several highly differentiated and well-supported phylogenetic lineages, possibly belonging to several distinct species. This study, for the first time, clearly showed five distinct clades found in Thailand with geographic overlap between Clades I and II, Clades II and III, and Clades IV and V. As well, the island populations in the Andaman Sea were included in Clade II, and also showed genetic difference between the distant islands (Adang and Rawi islands) and the island closer to the peninsular Thailand (Tarutao island). We propose that Peninsular Thailand acted as a center of isolation and diversification for three clades (Clades I, II, and III), and that subsequent dispersal events shaped their current complex and mosaic distribution with overlapping ranges in the Peninsular Thailand.

KEYWORDS: *Maxomys surifer*, peninsular Thailand, phylogeography, cytochrome *b*, Kra Isthmus

INTRODUCTION

Spiny rats (Genus *Maxomys*) are an endemic rodent species in Southeast Asia belonging to the family Murinae. The remarkable diversity, abundance, and broad distribution of mammals in the region make them an excellent subject for biogeographic investigation (Ruedas and Kirsch, 1997). Eighteen species are recognized in the genus *Maxomys*, with a general distribution stretching from mainland Southeast Asia eastward over the Sunda Shelf to some nearby islands (Achmadi et al., 2013). Sody (1936) proposed the genus *Maxomys* for *Rattus bartelsii* (originally named as *Mus bartelsii* [Jentink, 1879]), and Musser et al. (1979) later refined this definition by adding 4 species of *Leopoldamys* (at that time in the genus *Rattus*), *nativitatis*, *edwardsi*, *sabanus*, and *ohiensis*. Many authors have stated the need for improved information on species delimitation and phylogenetic relationships of the genus *Maxomys* (Musser, 1969; Musser et al., 1979; Ruedas and Kirsch, 1997; Gorog et al., 2004; Musser and Carleton, 2005; Achmadi et al., 2012).

Among 18 species of *Maxomys*, *M. surifer* (Miller, 1900) is the most widespread and has long been recognized to involve taxonomic problems. Considerable morphological variation and cryptic genetic divergence among populations across its broad range have raised questions about whether *M. surifer* represents a single species or a species complex (Ruedas and Kirsch, 1997; Achmadi et al., 2013). Diagnostic characters such as pelage coloration, spine density, and cranial morphology often overlap with those of closely related congeners (e.g., *M. whiteheadi*, *M. rajah*), leading to inconsistent identifications among regional populations. This species is found throughout Southeast Asia, including southern Myanmar, Thailand, Lao PDR, southern and southwestern Cambodia, Vietnam (including the Thom and Phu Quoc Islands off the south coast), extreme southern Yunnan, China, the Malay Peninsula, Borneo, Sumatra, Java, and many smaller islands (Musser and Carleton, 2005). *M. surifer* was originally described from Trang, Peninsular Thailand, by Miller (1900), and 46 taxa are currently considered junior synonyms of *M. surifer* as described between 1900 and 1940 by various researchers (Musser and Carleton, 2005).



FIGURE 1. External morphology of the Red Spiny Rat (*Maxomys surifer*), showing the characteristic reddish-brown spiny dorsal pelage, whitish ventral fur, and relatively long tail.

Maxomys surifer (Fig. 1) can be distinguished from the other *Maxomys* species by the fur thickly spiny, upperpart uniform tawny ochraceous, heavily sprinkled with blackish brown on the posterior half of back, less so on the shoulders and head and a tail about equal to the head and body, though usually somewhat longer, bicolor with the exception of the terminal third or fourth, which is entirely dull white (Miller, 1900). *M. surifer* can be found in lowland and highland forests up to 3,000 m above sea level, from primary forests, secondary forests, plantations, and disturbed areas close to forests (Achmadi, 2010). It feeds on plant material such as fruit, seed, and roots, as well as insects and small invertebrates. It lives in an underground burrow during the day (Achmadi, 2010; Wilson et al., 2017). This species is classified as Least Concern by the International Union for Conservation of Nature (IUCN Red List) because it is a prevalent species; it has a presumed large population, no major threats, and occurs in many protected areas (Aplin, 2016).

Latinne et al. (2013) reported the diversity and phylogeny of Murinae associated with limestone karst throughout Thailand based on a General Mixed Yule Coalescent (GMYC) model using an ultrametric tree. Within the *M. surifer* complex in the COI tree, six GMYC lineages (Max1 to Max6) were found in Thailand. Latinne et al. (2013) reconstructed a

maximum likelihood (ML) tree based on COI sequences of Thailand and southwestern Asian nationals. This ML tree reveals the GMYC lineages of Max1 and 2 of western and northern Thailand populations closely related to each other, Max3 of central and northeastern Thailand populations related to populations in Cambodia, Laos, and Vietnam, and Max4 to Max6 of Southern Thailand populations related to the Malaysian Peninsula population, and the Indonesian population and the Laos population were in a distinct clade. Latinne et al. (2013) generated three groups of four GMYC lineages (Max1 and 2, Max3, Max4, Max5 and 6) on the basis of 7.5% distance based on the genetic distance determined from the *Cytb* sequencing. Their divergent time was estimated as Max1 and 2 diverged from Max3 – 6 around 2.3 Mya; subsequently, Max3 diverged from Max4 – 6 around 2 Mya. These findings support Musser and Carleton's (2005) suggestion that *M. surifer* might be a complex comprising many species and strongly suggest that Thailand involves at least three well-supported groups (Max1 and 2, Max3, Max4, Max5 and 6). Of these, three lineages (Max4, Max5, Max6) of *M. surifer* have been reported in southern Thailand (Latinne et al., 2013), but phylogeography on the populations of *M. surifer* in southern Thailand is not well clarified.

In this study, we aim to reveal the phylogeography of *Maxomys surifer* in Thailand, focusing on populations from peninsular Thailand and the Andaman Sea islands. To address the limited sampling coverage of previous studies, we generated new cytochrome *b* sequences and combined them with published data to reconstruct phylogenetic trees and haplotype networks. This approach allows us to assess geographic variation and genetic structure across Thailand, providing new insights into the evolutionary history of *M. surifer* in this biogeographically complex region.

MATERIALS AND METHODS

Study area

Thailand is located across two biogeographical regions: the northern Indochinese province and the southern Sundaic province. (Lekagul and McNeely, 1988; Grey et al., 1994). The former province included the northern region of Thailand, South China, Vietnam, Laos, Cambodia, and Myanmar. On the other hand, the latter province included the southern portions of Thailand, Malaysia, Sumatra, Java, and Borneo. At the moment, the Kra Isthmus in peninsular Thailand, north to Tavoy on the Myanmar side (14°N), and the Chumphon-Prachuap border (11°N) on the Thai side, is presumed to mark the border between the Indochinese province and the Sundaic province (Tougaard, 2001).

Thailand has been selected as the study area in this study. For the molecular investigation, fifty-nine new specimens from 24 localities were sequenced, and 33 sequences from GenBank were incorporated into the analyses. The locations and specimen list are displayed in Fig. 2 and Supplementary material 1.

Specimen and sampling

The specimens were collected in the forest in Peninsular Thailand by live traps, either of metal mesh traps (12 x 12 x 27 cm) or Sherman traps (7.6 x 8.9 x 22.9 cm). The live traps were set before sunset, left overnight, and checked the next morning. The target animals were collected for voucher specimens. For the external morphology, we weighed body mass (using the Pesola Spring scale, 600 grams, to the nearest 5 grams) and measured head and body length, tail length, ear length, and hind foot length by digital caliper (to the nearest 0.01 mm). The photographs of specimens were taken from several angles. The specimens were euthanized by Isoflurane C₃H₂ClF₅O (approved by the Federal Drug Administration – FDA 2023) in accordance with approved animal care guidelines (permit no. U1063472560, National Research Council of Thailand). Following euthanasia, the skin and skull

were prepared for reference collection, and voucher specimens were deposited in the Zoological collection at Princess Maha Chakri Sirindhorn Natural History Museum, Prince of Songkla University, Thailand. Small pieces of liver and muscle were collected and preserved in 99% or absolute ethanol for molecular study.

DNA Analysis

Genomic DNA was extracted using the DNeasy blood and tissue kit (QIAGEN Group, Hilden, Germany). Mitochondrial fragments from the cytochrome *b* region were amplified by polymerase chain reaction (PCR) using primer: MaxoFw (5'-TCCTTCATTGACCTACCYAC-3') and MaxoRv (5'-ATGATTGAGAAGTAGCTGATG-3') (Latinne et al., 2013). Cytochrome *b* PCR amplifications were performed in a 10 µl reaction volume using an AllTaq Master Mix Kit (QIAGEN) and a PCR Thermal Cycler Dice Gradient (SimpliAmp Thermal Cycler), with the following program: an initial denaturing step at 94.0°C for 4 min; 40 cycles of denaturing at 94.0°C for 30 s, annealing at 50.0°C for 60 s, and extension at 72.0°C for 90 s; and a final extension step at 72.0°C for 10 mins. The PCR products were purified and sequenced by Macrogen South Korea Co. The sequences were edited and trimmed using Bioedit version 7.2.5 (Hall, 1999), and sequences were aligned using ClustalW (Thompson et al., 1994) in MEGA11 version 11.0.13 (Tamura et al., 2021), and alignments were carefully inspected and adjusted manually where necessary.

Phylogenetic inference using Maximum Likelihood + rapid bootstrap approach was performed with raxmlGUI 2.0 (Edler et al., 2021) with branch support values evaluated by 1000 replicates of bootstrap probability (BP), GTR substitution matrix, and GAMMA substitution rates, the raxmlHPC-PTHREADS-SSE3 binary model, and 2 threads were set for analyses. The original phylogenetic trees (ML tree) were reconstructed by raxML as a distance matrix, then visualized by FigTree version 1.4.4 (Rambaut, 2018), and individuals were gathered into unique haplotypes. The haplotype diversity (*h*) and nucleotide diversity (π) of the cytochrome *b* were calculated in DnaSP version 6.12.03 (Rozas et al., 2017). Haplotype network construction was performed by a median-joining method in Popart version 1.7 (Bandelt et al., 1999). Averages of uncorrected *p*-distances were calculated in MEGA11 version 11.0.13 (Tamura et al., 2021), both within a group and between groups.

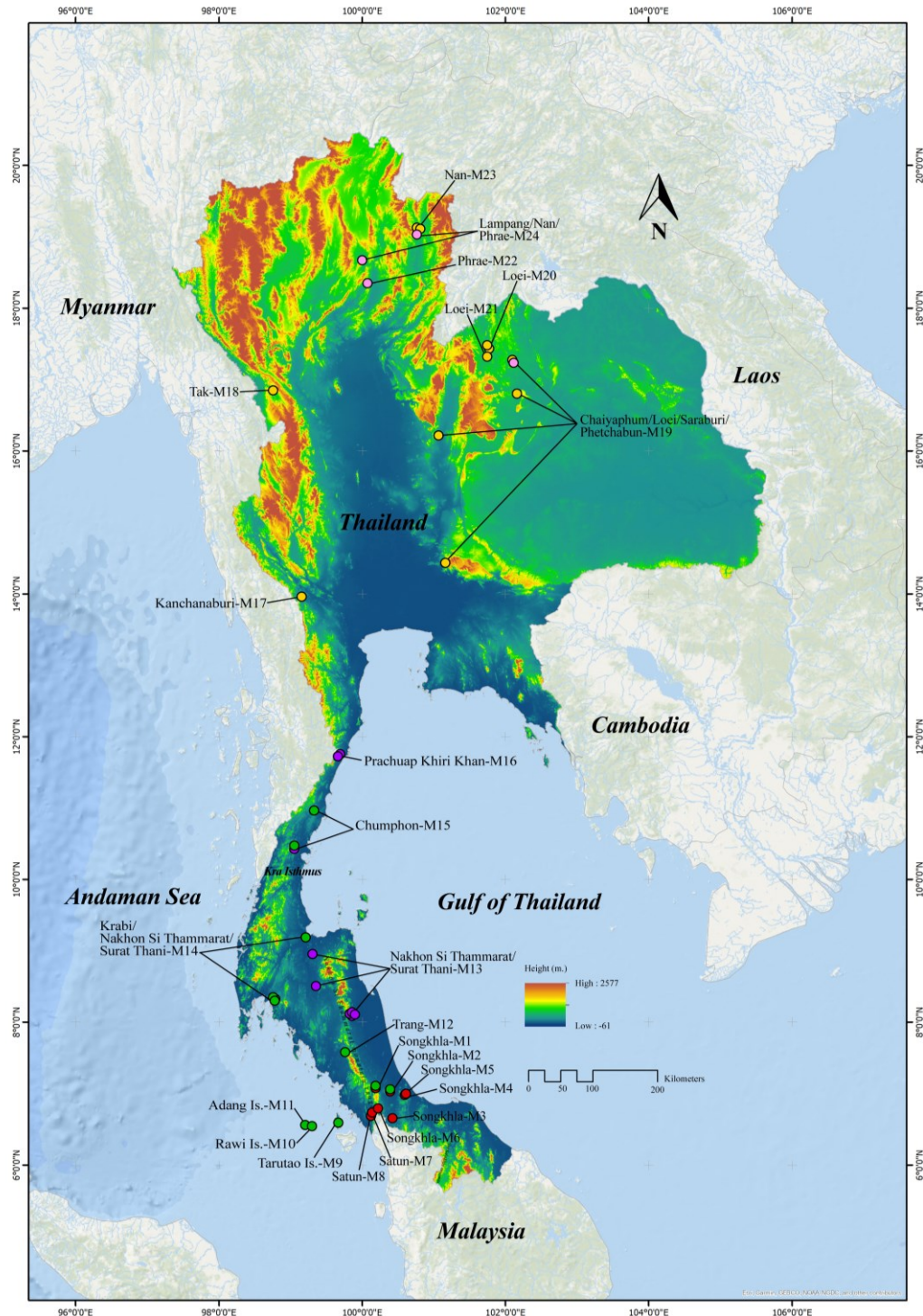


FIGURE 2. Map of Thailand showing the localities of the samples examined in this study: Clade I (red circle), Clade II (green circle), Clade III (purple circle), Clade IV (yellow circle), and Clade V (pink circle). M prefix is the place (location) number. (Map information: <https://earthexplorer.usgs.gov/> and <https://server.arcgisonline.com/ArcGIS/services> (5 June, 2024))

RESULTS

A total of 92 individuals of *Maxomys surifer*, comprising 59 new sequences (accession no. PQ424736–PQ424794) and including 33 GenBank sequences of the previously deposited data as well, were included to determine the cytochrome *b* (*Cytb*) with 898 base pairs (bp).

We recovered a total of 66 unique haplotypes are shown in the study, and the phylogenetic tree using maximum likelihood revealed five highly divergent and well-supported lineages (Fig. 3, Supplementary material 1). The geographic distribution of haplotypes is presented in Fig. 2: Clade I, specimens from a small area of Songkhla and Satun provinces in peninsular Thailand; Clade II, specimens from Songkhla, Trang,

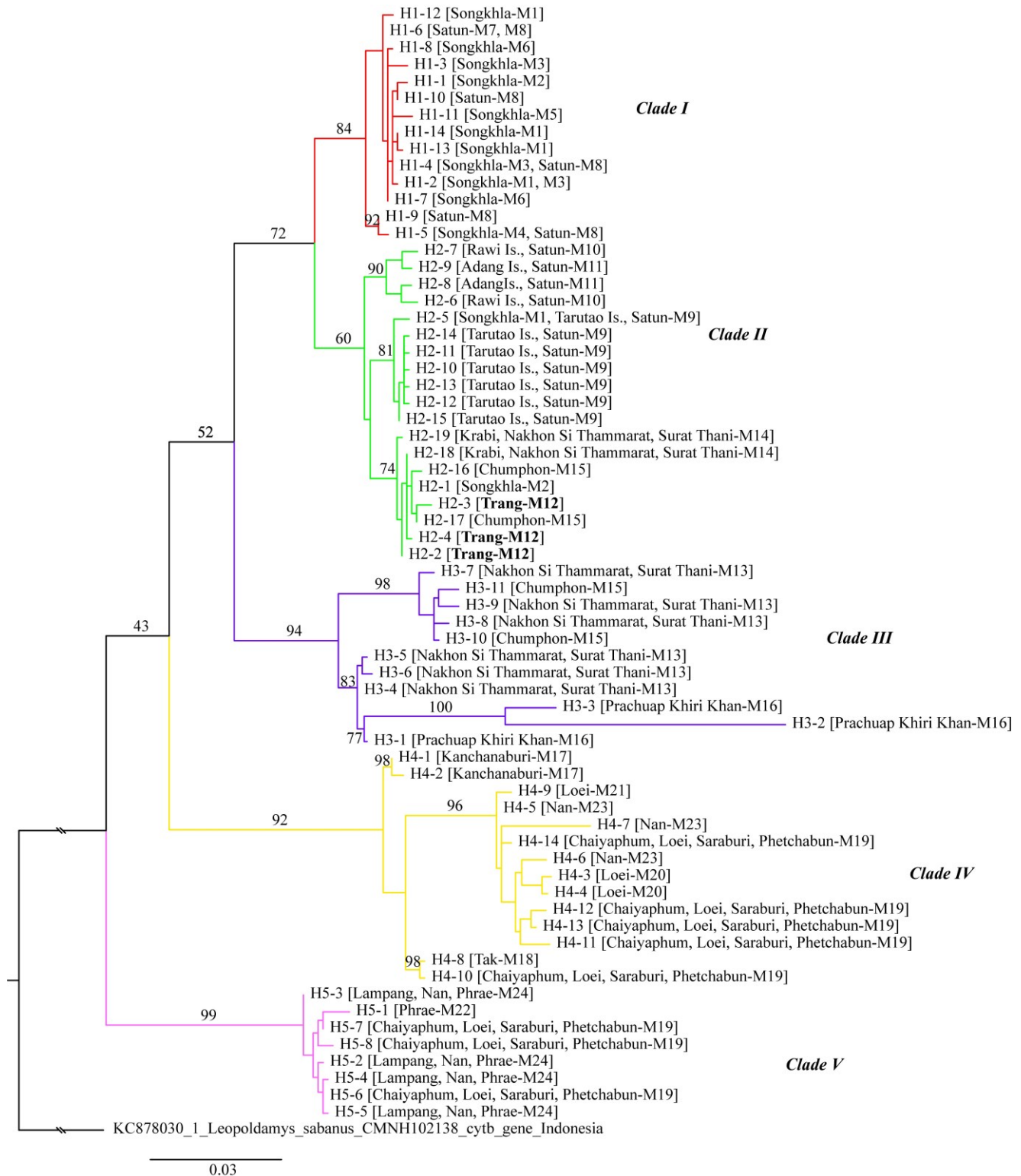


FIGURE 3. Phylogenetic tree of *Maxomys surifer* with maximum likelihood bootstrap probability. Haplotypes were named with the lineage name (“H1-” for the Clade I, “H2-” for the Clade II, “H3-” for the Clade III, “H4-” for the Clade IV and “H5-” for the Clade V) followed by the consecutive numbers in each lineage. Red color, Clade I; green color, Clade II; purple color, Clade III; yellow color, Clade IV and pink color, Clade V. Haplotypes of specimens from the same location of type locality (in Trang) are shown in bold.

Nakhon Si Thammarat, Krabi, Surat Thani and Chumphon provinces in peninsular Thailand and three islands (Adang Is., Rawi Is., Tarutao Is.) in the Andaman Sea; Clade III, specimens from Nakhon Si

Thammarat, Surat Thani, and Chumphon provinces in peninsular Thailand and Prachuap Khiri Khan in the area near Kra Isthmus; Clade IV, specimens from the Indochinese subregion from Kanchanaburi and Tak

TABLE 1. Number of haplotypes, haplotype diversity and nucleotide diversity of Clade I, Clade II, Clade III, Clade IV and Clade V based on mitochondrial cytochrome *b*.

Clade	n	nh	π	h
Clade I	21	14	0.00583	0.957
Clade II	34	19	0.01231	0.943
Clade III	11	11	0.03774	1.000
Clade IV	17	14	0.01690	0.978
Clade V	9	8	0.00512	0.972
All clades	92	66	0.04197	0.984

Abbreviations: n, number of individuals; nh, number of haplotypes; h, haplotype diversity; π , nucleotide diversity

TABLE 2. Pairwise uncorrected p-distances between Clade I, Clade II, Clade III, Clade IV and Clade V based on mitochondrial cytochrome *b*. The shaded cells represent the genetic distances within each clade.

Clade	Clade I	Clade II	Clade III	Clade IV	Clade V
Clade I	0.60				
Clade II	3.27	1.26			
Clade III	6.79	6.80	3.77		
Clade IV	8.15	7.75	9.69	1.91	
Clade V	8.60	8.34	9.15	9.67	0.52

provinces in the western Thailand, Saraburi and Phetchabun provinces in the central Thailand, Loei and Chaiyaphum provinces in the northeastern Thailand, and Nan province in the northern Thailand; Clade V, the specimens from the Indochinese subregion from Saraburi and Phetchabun provinces in the central Thailand, Loei and Chaiyaphum provinces in the northeastern Thailand, and Lampang, Nan and Phrae provinces in the northern Thailand.

Latitudinal distribution ranges of each clade in Thailand were as follows: Clade I, 6.65°N-7.06°N; Clade II, 6.54°N-10.97°N; Clade III, 8.17°N-11.78°N; Clade IV, 14.13°N-19.14°N and Clade V, 14.62°N-19.18°N. There was no overlap between the southern clades (Clades I, II, and III) and the northern clades (Clades IV and V). Clades I and II overlap in Songkhla province in the peninsular Thailand; Clades II and III both were found in Chumphon province in the peninsular Thailand; Clades IV and V were both found in the same locality in Loei province in the northeastern Thailand and Nan province in the northern Thailand (Fig. 2).

Sixty-six haplotypes were detected in Thailand with a haplotype diversity of 0.984 and nucleotide diversity of 0.04197 (Table 1). Haplotype diversity and nucleotide diversity in each clade were 0.943-1.000 and 0.00512-0.03774, respectively. Nucleotide diversity was high in Clade III (0.03774) compared with the other four clades (0.00512–0.01690). The genetic distance within each clade was 0.60–3.77% (0.60% in Clade I, 1.26% in Clade II, 3.77% in Clade III, 1.91% in Clade IV, and 0.52% in Clade V), while pairwise genetic distances among clades were from 3.27% (Clades I and II) to 9.69% (Clades III and IV, Table 2).

Clade I was supported by an ML bootstrap value of 84, comprising 21 sequences with 14 haplotypes. The haplotype diversity was 0.957, and the nucleotide diversity was 0.00583 (Table 1). Haplotypes H1-1, H1-2, H1-3, H1-4, H1-5, H1-7, H1-8, H1-13, and H1-14 were from Songkhla, while haplotypes H1-4, H1-6, H1-9, and H1-10 were from Satun (a province near Songkhla), and those haplotypes were closely clustered with low genetic divergence and did not show any

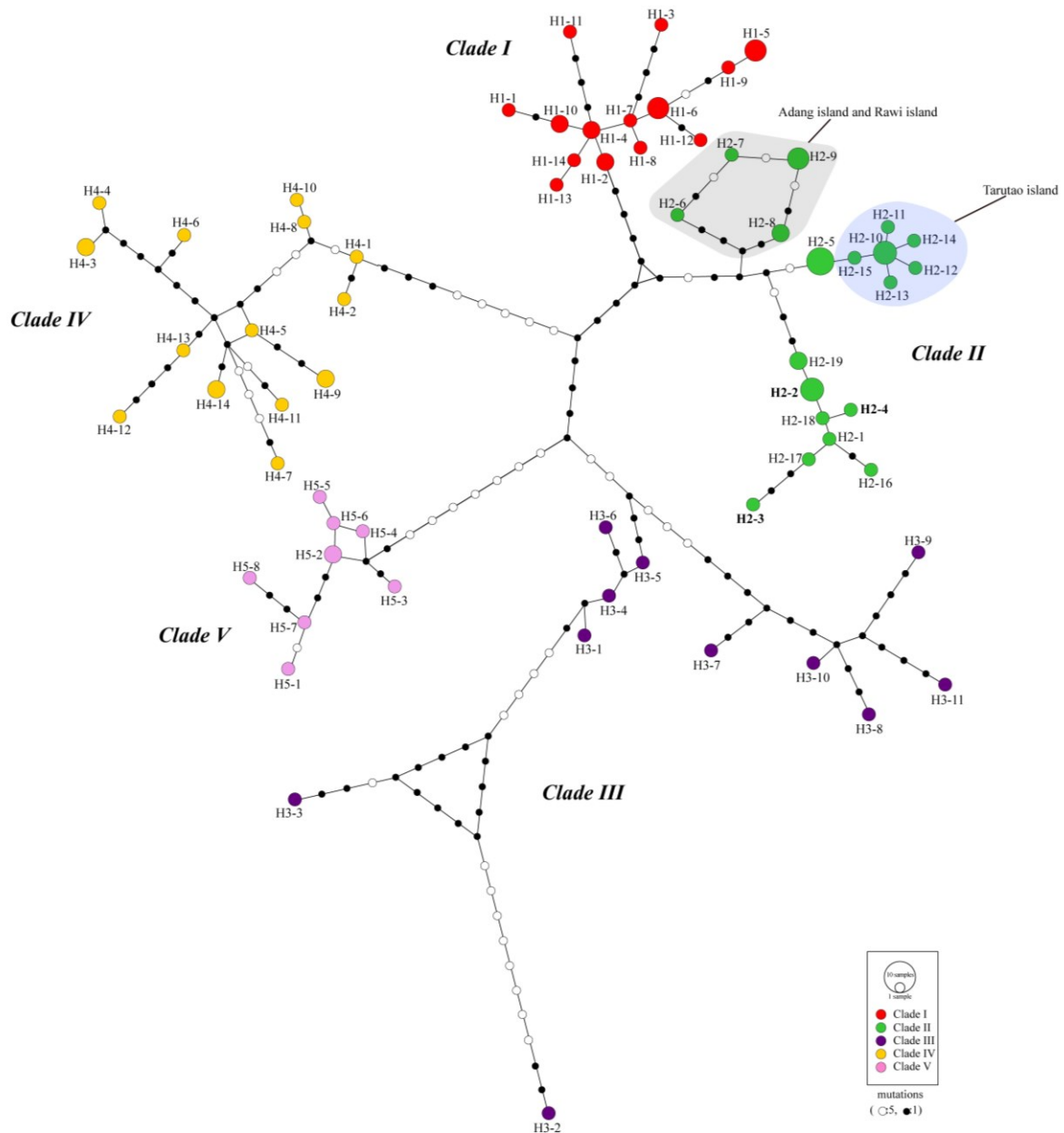


FIGURE 4. Haplotype networks of *Maxomys surifer* (Clades I–V). Sets of haplotypes are represented by red, green, purple, yellow, and pink dots, whereas grey and blue backgrounds indicate geographic groupings (e.g., islands or populations) as explained in the text. Haplotypes were named according to their clade (“H1–” for Clade I, “H2–” for Clade II, and “H3–” for Clade III), followed by consecutive numbers within each lineage.

geographic pattern in the haplotype network, as shown in Fig. 4.

Clade II was supported by an ML bootstrap value of 60, comprising 34 sequences with 19 haplotypes. The haplotype diversity was 0.943, and the nucleotide diversity was 0.01231 (Table 1). Clade II could be divided into three subclades in phylogenetic tree and haplotype network: 1) specimens from Adang (H2-8, H2-9) and Rawi (H2-6, H2-7) islands in the Andaman Sea (approx. 70–80 km from the mainland) without difference between Adang and Rawi islands (Figs. 3, 4), 2) specimens from Tarutao island (H2-5, H2-10, H2-

11, H2-12, H2-13, H2-14, H2-15) in the Andaman Sea (approx. 26 km from the mainland) in sharing a haplotype (H2-5) with a specimen from Khuan Khao Wang, Songkhla (M1) in peninsular Thailand, and 3) specimens from the mainland of peninsular Thailand, including H2-1 (Songkhla), H2-2 (Trang, Krabi, Nakhon Si Thammarat, Surat Thani, and Chumphon), H2-3 and H2-4 (Trang), H2-16 and H2-17 (Chumphon), and H2-18 and H2-19 (Krabi, Nakhon Si Thammarat, and Surat Thani) as shown in Fig. 4. Bold letters indicate the haplotypes, including specimens collected from Trang, the type locality of *M. surifer*.

Clade III was supported by an ML bootstrap value of 94, comprising 11 sequences with 11 haplotypes. The haplotype diversity was 1.000, and the nucleotide diversity was 0.03774 (Table 1). The locality of each haplotype is the middle to the northern Kra Isthmus: H3-1, H3-2, and H3-3 were from Prachuap Khiri Khan, H3-4, H3-5, H3-6, H3-7, H3-8, and H3-9 were from Nakhon Si Thammarat and Surat Thani, and H3-10 and H3-11 were from Chumphon, as shown in Fig. 4. Three haplotypes from Prachuap Khiri Khan (H3-1, H3-2, H3-3) showed extensive divergence from each other (Figs. 3, 4).

In contrast that Clades I, II, and III were found in the Sundaic subregion and the area close to Kra Isthmus, Clades IV and V consisted of specimens from the Indochinese subregion. Clade IV was supported by an ML bootstrap value of 92 and comprised of 17 sequences with 14 haplotypes; the haplotype diversity was 0.978, and the nucleotide diversity was 0.01690 (Table 1). Clade IV was found in the central, western, northern, and northeastern Thailand: H4-1 and H4-2 from Kanchanaburi in western Thailand; H4-3, H4-4 and H4-9 from Loei in northeastern Thailand; H4-5, H4-6 and H4-7 from Nan in northern Thailand; H4-8 from Tak in western Thailand; and H4-10, H4-11, H4-12, H4-13, H4-14 from Chaiphaphum, Loei, Saraburi, Phetchabun in central, northern and northeastern Thailand. Clade V was supported by an ML bootstrap value of 99, comprising 9 sequences with 8 haplotypes; the haplotype diversity was 0.972, and the nucleotide diversity was 0.00512 (Table 1). Clade V was found in the northern and northeastern Thailand: H5-1 from Phrae in northern Thailand; H5-2, H5-3, H5-4, and H5-5 from Lampang, Nan, Phrae in northern Thailand; H5-6, H5-7, and H5-8 from Chaiphaphum, Loei, Saraburi, Phetchabun in northeastern Thailand

DISCUSSION

The phylogenetic tree (Fig. 3) showed that *M. surifer* from Thailand consists of five highly divergent lineages: Clade I, Clade II, Clade III, Clade IV, and Clade V. Clades IV and V are found exclusively in the Indochinese region, while Clades I, II, and III occur mostly in the Sundaic region. Latinne et al. (2013) discussed that *M. surifer* comprises several highly differentiated and well-supported phylogenetic lineages possibly belonging to several distinct species in Thailand. The reconstruction of the ML phylogenetic tree of *M. surifer* in the present study showed that there is considerable genetic variation between Clade I – V, suggesting that it may correspond to different species of

“*M. surifer*” in Thailand, and the geographic overlapping between several pairs of clades (Clades I and II, Clades II and III, Clades IV and V). Three clades from peninsular Thailand (Clades I, II, and III, as bold in Fig. 3) are clearly separated genetically and geographically from the central, western, northern, and northeastern Thailand populations.

The explanations of the separation between the above and lower Kra Isthmus may be responsible for the location at the intersection of the Indochinese and Sundaic subregions, the two main biogeographical regions of Southeast Asia. Climate, floristry, and fauna, these subregions are unique and distinct from each other. The Kra Isthmus in peninsular Thailand forms the current border between the Indochinese and Sundaic subregions (Tougaard, 2001) in many animals, including arthropods (de Bruyn et al., 2005; Thummajitsakul et al., 2008; Jantarit et al., 2013), reptiles (Inger and Voris, 2001), bats (Soisook et al., 2008; Dejtaradol, 2009), and plants (Woodruff, 2003). *M. surifer* also might have experienced a long time of isolation between the Indochinese and Sundaic subregion between the above and lower Kra Isthmus.

The occurrence of Clades IV and V in the Indochinese subregion, and their overlapping distribution almost concordant with Latinne et al. (2013). Clade V is found from 14.13°N, 99.19°E to 19.14°N, 100.72°E, in Kanchanaburi and Tak provinces in western Thailand, Saraburi and Phetchabun provinces in central Thailand, Loei and Chaiphaphum provinces in northeastern Thailand, and Nan province in northern Thailand. While Clade V is located from 17.30°N, 101.93°E to 19.18°N, 100.72°E, in Saraburi and Phetchabun provinces in the central Thailand, Loei and Chaiphaphum provinces in the northeastern Thailand, and Lampang, Nan, and Phrae provinces in northern Thailand. The result in this study supported previous studies; there is more than one taxon of *M. surifer* in the Indochinese subregion (Latinne et al., 2013; Balakirev et al., 2017).

Latinne et al., (2013) suggested that *M. surifer* in peninsular Thailand is separated from *M. surifer* from central + northern + northeastern Thailand by a genetic distance of more than 7.5%, which is similar with a genetic distance of this study (Table 2), where the genetic distance between Clades I – III and Clades IV – V is 7.75–9.69%. In this study, Clades I, II, and III were found in peninsular Thailand, and the genetic distance between Clades I – III (3.27–6.80%) potentially represents species-level divergences within *M. surifer* in peninsular Thailand; additional morphological, ecological, and nuclear genetic data would be required to confirm their taxonomic distinctiveness.

The present study focused on peninsular Thailand populations with more specimens compared with the previous studies provided evidence of more complicated divergence of *M. surifer* in Thailand. Latinne et al. (2013) only found 2 well-supported clades based on 17 specimens in peninsular Thailand, while we found 3 clades from peninsular Thailand and the islands in the Andaman Sea based on 66 specimens, with the first finding of our Clade I with restricted distribution in Songkhla and Satun provinces in the east of the Nakhon Si Thammarat mountain range. As well, the distribution pattern of each clade in southern Thailand was not simply separated geographically, and more complicated and overlapping. As well, we investigated the dispersal and origin of the island populations in the Andaman Sea and their inclusion in the Clade II found in peninsular Thailand.

Clade I included specimens from the small area of Songkhla and Satun provinces (Fig. 5) located close to southernmost peninsular Thailand and was supported as a distinct monophyletic group by an ML bootstrap value of 84. No previous papers have analyzed specimens from this location for phylogenetic analysis. Thus, this study first revealed the existence of a highly diverged Clade I restricted to this small area. Specimens of Clade I were collected on the eastern side of the Nakhon Si Thammarat mountain range (approximately 6.4°N–9.1°N, 460 km NW–SE in length and 49 km NE–SW in width; Travel News, 2008), which separates the western Satun from the eastern Satun and Songkhla provinces. This mountain range may have acted as a geographical barrier limiting the dispersal of Clade I into western Satun, leading to its divergence from Clade II, with which it forms a sister relationship. However, the proposed barrier effect remains hypothetical, as the timing of the mountain uplift and potential historical dispersal events (e.g., possible eastward expansion of Clade II) are not yet well understood. Further geological and biogeographic evidence from other taxa would help to support this scenario.

Clades I and II show largely separate distributions on the west and east of the Nakhon Si Thammarat mountain range, although both clades occur sympatrically in northeastern localities (M1, M2).

Clade II was distributed widely covering the area of the peninsular Thailand and islands in the Andaman Sea from the northern isthmus of Kra to Songkhla provinces (Fig. 5), near the border of Malay peninsula, and divided into three subclades found in this study: subclade 1, specimens from Adang and Rawi islands (close to coordinates; 6.54°N, 99.29°E and 6.56°N, 99.22°E, respectively, and approx. 70–80 km off the mainland), with ML bootstrap value of 90; subclade 2,

specimens from Tarutao island, approx. 26 km off the mainland, as well as from Songkhla in the mainland of peninsular Thailand with ML bootstrap value 81; and subclade 3, specimens from the mainland of peninsular Thailand, including specimens from Kachong in Trang province (the type locality of *M. surifer*), Songkhla, Nakhon Si Thammarat, Krabi, Surat Thani, Chumphon, with ML bootstrap value of 74. Relationships among the 3 subclades of Clade II were uncertain.

Within Clade II, island populations were grouped into distinct subclades, suggesting at least two independent colonization events from peninsular Thailand: subclade 1 includes specimens from the distant Adang and Rawi Islands, and subclade 2 includes those from the nearer Tarutao Island. Specimens from the type locality of *M. surifer* (M12) were assigned to subclade 3 (Fig. 5). To clarify this pattern, the subclade structure and corresponding island localities are highlighted in Fig. 5.

The northern limit of Clade II in peninsular Thailand was Chumphon (M15), located approximately 36 km north of the Kra Isthmus, while the southernmost localities were in Songkhla province (M1, M2). The Songkhla province is separated from the Malay Peninsula by the San Kala Khiri Mountain (143 km²) and Khao Nam Kang Mountain (212 km²) (Department of National Parks, 2022, 2023). The phylogeographic relationship of *M. surifer* populations on the Malay side remains uncertain and requires further study.

Clade III consisted of the specimens with ML bootstrap value 94 from the mainland of peninsular Thailand: Nakhon Si Thammarat, Surat Thani, Chumphon, and Prachuap Khiri Khan provinces (Fig. 5). In this clade, Prachuap Khiri Khan is approximately 88 km north of the center point of Kra Isthmus, and represents the northernmost province (11.78°N, 99.68°E) of the Clade III, it is noted that the three haplotypes from this northernmost province showed extensive divergence, and the reason for forming such diversity is unclear. On the other hand, the southernmost distribution of Clade III is Nakhon Si Thammarat province (8.17°N, 99.81°E). Latitudinal distribution is supposed to be widely overlapping between Clades II and III, while the actual pattern of overlapping and segregation of the two clades could not be clarified in this study.

Even though 3 clades in southern Thailand show different distribution range as Clade I in the south, Clade II in the middle, and Clade III in the north. There are overlapping in some localities between Clades I and II, and Clades II and III. These may suggest that the peninsular Thailand has functioned as an important speciation place. Three clades have been formed by the

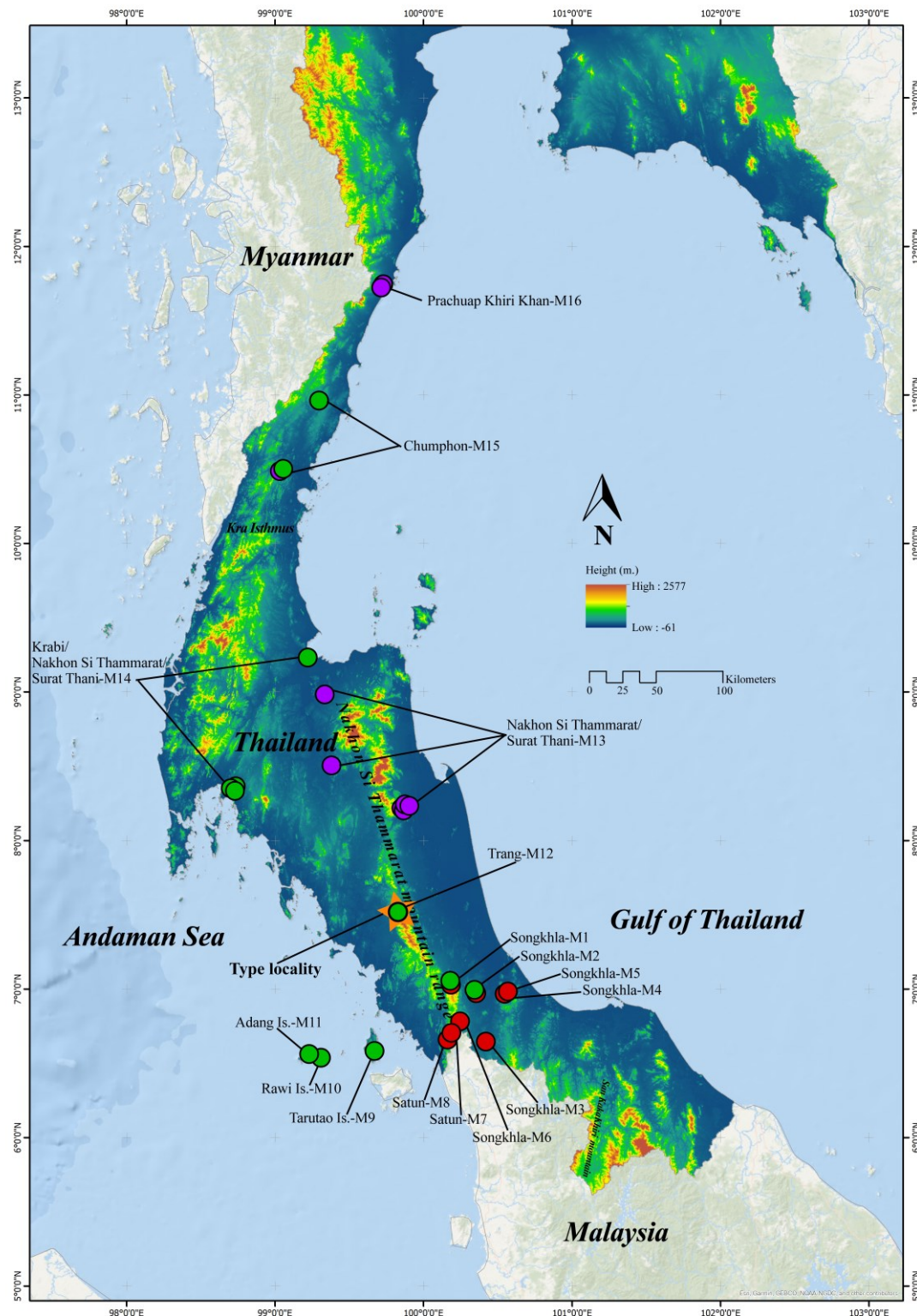


FIGURE 5. The Map of peninsular Thailand showing the distribution of Clade I (red circle), Clade II (green circle) and Clade III (purple circle), the orange star shows the type locality of *M. surifer* (Miller, 1900). M prefix is the place (location) number. (Map information: <https://earthexplorer.usgs.gov/> and <https://server.arcgisonline.com/ArcGIS/services> (5 June, 2024))

geographic isolation such as the high mountains of the Nahkon Si Thammarat mountain range, and made a divergence among clades. The complicated topography and very narrow and small area may have accelerated the divergence and speciation in forming the small isolated populations. Subsequent climate change made

each clade expansion, mainly in the north-south direction, and secondary contact between Clades I and II, and Clades II and III occurred and formed the current mosaic distribution of the 3 clades in southern Thailand.

Moreover, island populations in the Andaman Sea become clear in this study to have derived from the peninsular Thailand Clade II, and interestingly, the far islands (Adang Is. and Rawi Is.) and the closer island (Tarutao Is.) are located in different subclades, suggesting different times and origins of dispersals from the peninsular Thailand. Miller (1900) treated the specimens from Adang and Rawi islands as a subspecies of *M. s. butangensis* and Chasen (1940) considered the specimens from Tarutao Island as *M. s. flavidulus* both based on the morphological differences of pelage coloration and tail length. Phylogeographic and morphological examination of islands of the Andaman Sea in Thailand and Malaysia should be carried out to answer the taxonomic status of island populations of *M. surifer*.

In taxonomy, our study strongly suggested that the real *M. surifer* belongs to Clade II in this study, based on the haplotype of specimens from the type locality ("Trong", now called "Trang"), where only Clade II is found. To clarify the taxonomic name of five clades in Thailand, it is expected that a comprehensive taxonomic study of this widely distributed species, "*M. surifer*", in the Indochinese and Sundaic regions will be conducted.

In summary, our study provides new insights into the phylogeography of *Maxomys surifer* in peninsular Thailand and the Andaman Sea islands, revealing complex patterns of divergence, isolation, and secondary contact among clades. These findings highlight the roles of geographic barriers and historical dispersal in shaping the species' evolutionary history. Further studies integrating morphological, ecological, and nuclear genetic data are needed to clarify the taxonomy and evolutionary relationships of *M. surifer* across Southeast Asia.

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