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RESEARCH ARTICLE

High genetic diversity and cryptic lineages in three *Tabanus* species (Diptera: Tabanidae) from Northeastern Thailand

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ABSTRACT

Species of the genus *Tabanus* Linnaeus (horse flies) are significant pests and vectors transmitting pathogens to economically important animals including cattle. Therefore, knowledge about genetic diversity; a necessity for understanding disease epidemiology, is of paramount importance. However, such information is limited in Thailand, particularly in the northeastern region despite it being a major cattle production area. We examined genetic diversity of three *Tabanus* species, *T. striatus* Fabricius and *T. megalops* Walker, and *T. rubidus* Wiedemann based on mitochondrial cytochrome oxidase I (COI) sequences. There was relatively high genetic diversity (max. 2.14%) within populations of *T. rubidus* from northeastern Thailand but no indication of genetic structuring. Phylogenetic relationships between Thai populations and those recorded from Malaysia, India and Bangladesh involved three genetically divergent lineages which possibly represent different but morphologically similar species. High levels of genetic diversity (max. 4.43% and 5.20%) were found within northeastern populations of *T. megalops* and *T. striatus* due to the existence of three genetically divergent lineages within these species. However, there was no signal of geographical association of lineages suggesting panmixia within populations. Phylogenetic relationships between specimens obtained in the present study and those recorded from other countries revealed cryptic genetic diversity with three divergent clades. Further study is required to solve this taxonomic complexity of the *T. striatus*-complex.

Keywords: Insect vectors; COI; genetic variation; *Tabanus*.

INTRODUCTION

The genus *Tabanus* Linnaeus comprises approximately 1,300 species (Morita *et al*., 2016), many of which are significant pests and vectors which can transmit to humans and other animals a diverse range of pathogens including viruses, bacteria and protozoa (Mullens, 2019). Significant diseases for which *Tabanus* species act as vectors of the disease agents include equine infectious anemia, bovine leukemia, hog cholera, Anaplasmosis, Elaeophorosis and surra (Baldacchino *et al*., 2014).

Approximately 80 species of the genus *Tabanus* have been recorded in Thailand (Burton, 1978). Among these, *T. striatus* Fabricius and *T. megalops* Walker, and *T. rubidus* Wiedemann were considered the most abundant (Changbunjong *et al*., 2018a). *Tabanus rubidus* is a geographically widespread and common species being recorded from western Pakistan to China, mainland Southeast Asia to Indonesia (Burton, 1978). In Thailand, this species has been recorded throughout the country (Changbunjong *et al*., 2018a) and was considered a pest due to its high abundance on cattle farms (Burton, 1978; Phasuk *et al*., 2011). *Tabanus rubidus* is also a vector of pathogens including *Clostridium chauvoei* and *Bacillus anthracis* (Baldacchino *et al*., 2014) and *Trypanosoma evansi* (Gill, 1977; Desquesnes *et al*., 2013). *Tabanus striatus* Fabricius and *T. megalops* Walker are members of the *T. striatus* complex that have been found in Thailand (Burton, 1978). These species are morphologically similar (Burton, 1978) and genetically closely related (Changbunjong *et al*., 2018b). Molecular studies found diverse pathogens including *Anaplasma*, *Ehrlichia*, *Babesia*, and *Theileria* in *T. megalops* from southern Thailand (Sontigun *et al*., 2022; Phetkarl *et al*., 2023). These findings indicate the possibility of *T. megalops* as a significant vector of pathogens causing disease in cattle. *T. striatus* has been determined to be a vector of *Trypanosoma evansi*, the causal agent of surra (Gill, 1977).

Knowledge about genetic diversity of the vector species is important for understanding disease epidemiology and determining effective control strategies (Tabachnick & Black, 1995; McCoy, 2008;

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Beaurepaire *et al*., 2024). However, information of genetic diversity of *Tabanus* species in Thailand is scanty. Thus far, studies have been limited to DNA barcoding using mitochondrial cytochrome oxidase I (COI) sequences (Changbunjong *et al*., 2018b). DNA barcoding of the horse flies in Thailand found that the majority of species examined had relatively low (<2%) intraspecific genetic divergence. This low level of genetic diversity was possibly a result of small sample size (43 of 48 studied species had sample size <5) although specimens were collected from many locations throughout Thailand. However, there was very limited information from the northeastern area with only two, one and three specimens for *T. rubidus*, *T. megalops*, and *T. striatus*, respectively (Changbunjong *et al*., 2018b). Despite being limited by small sample size, high genetic diversities (>4%) and cryptic genetic divergent lineages were found in some species including *T. megalops* (Changbunjong *et al*., 2018b). Therefore, additional genetic diversity could be expected by including specimens from undersampled areas such as the northeastern region where these species are found as common pests of the cattle (Thinnabut *et al*., 2024).

The northeastern region of Thailand is an important location for cattle production with more than 56% of the National Herd of cow and buffalo in this area (Department of Livestock Development, 2025). Because *Tabanus* species are significant pests and vectors of disease in cattle (Baldacchino *et al*., 2014), understanding their genetic diversity is of particular importance because it can be related to the vector-pathogen competence (McCoy, 2008; Powell, 2018). For example, distinct genetic lineages of the mosquito *Aedes albopictus* Skuse, resulting from historical isolation and divergence, had varied competences for the Chikungunya virus (Vega-Rúa *et al*., 2020).

In this study, we examined genetic diversity of three *Tabanus* species: *T. rubidus*, *T. striatus* and *T. megalops* in northeastern Thailand. We also investigated genetic relationships between Thai populations of these species with those reported from other countries using available COI sequences in public databases (i.e., GenBank and BOLD). Geographically widely distributed species experience different environmental conditions that could be driving genetic differentiation (Adler & McCreadie, 1997). Many studies have found that insect species distributed in geographically distant countries comprise potentially different species such as a deer fly (*Chryspos dispar* Fabricius) (Gomontean *et al*., 2024) and biting midge (*Culicoides arakawae* (Arakawa)) (Pramual *et al*., 2021). Therefore, it is important to examine the level of genetic of differentiation which can be used to infer species status of large geographically fragmented populations (Hajibabaei *et al*., 2007).

MATERIALS AND METHODS

Specimen collection and identification

Specimens of three *Tabanus* species were collected from 21 sampling sites in ten provinces of the Northeastern region of Thailand between May 2022 and August 2024 (Figure 1 and Table 1). Adult flies were collected using a sweep net swept around the area of the cattle pens. In addition, *Tabanus* flies were also collected by hand while the fly was attached to cattle. Specimens were preserved in 80% ethanol and stored at -20° C until investigation. Species were identified using the taxonomic keys for Tabanidae in Thailand provided by Burton (1978). Key morphological characters used for species identification included head structure, callus characteristics,

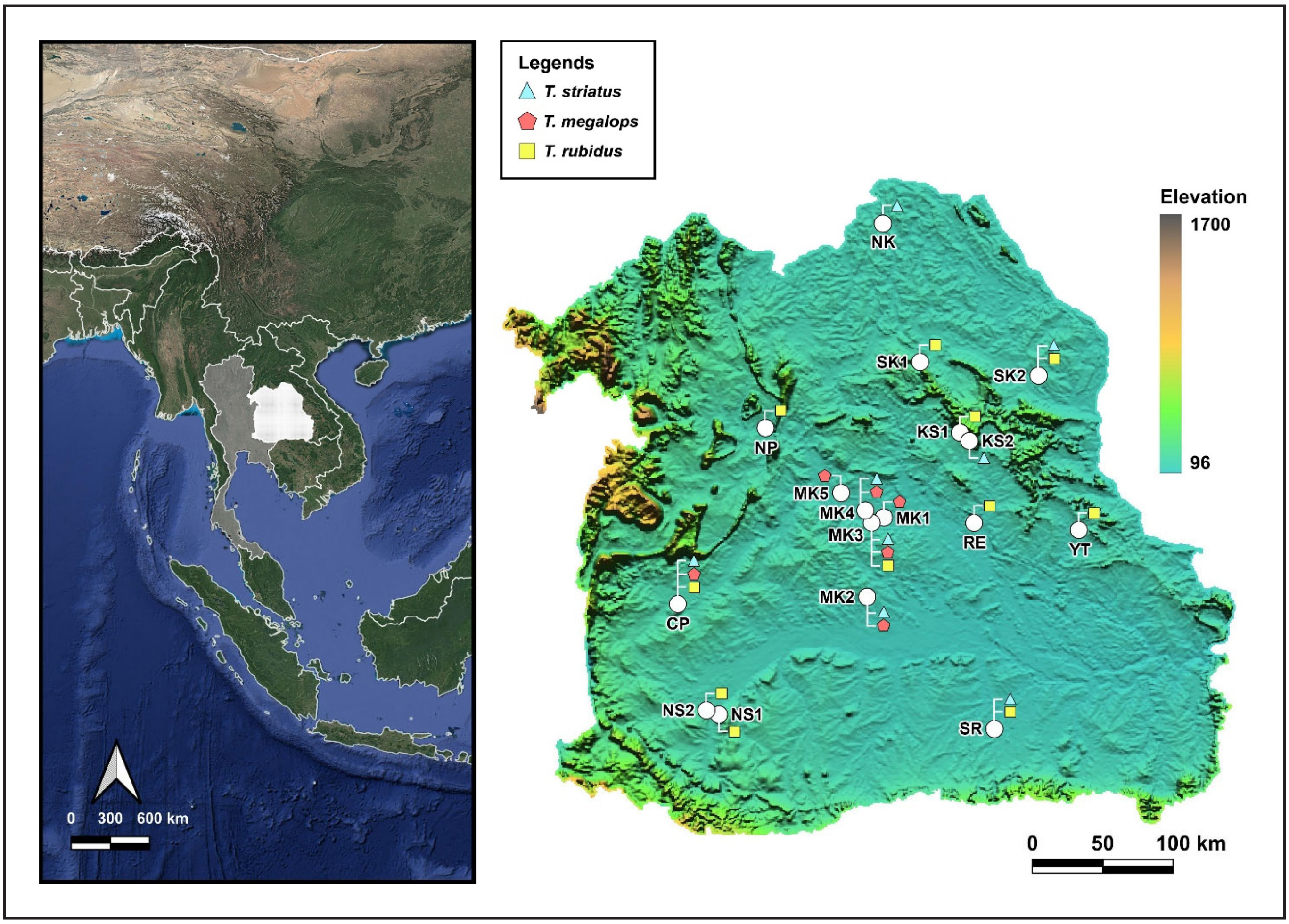

Figure 1. Sampling locations of *Tabanus rubidus*, *T. striatus* and *T. megalops* from northeastern Thailand. Details of each sampling site are provided in Table 1.

Table 1. Sampling locations and number of specimens of three horse fly species, *Tabanus rubidus, T. striatus* and *T. megalops* from northeastern Thailand used in this study

Location (code)	Species	N	Coordinates	Elevation (m)	Collection date
1. Rattanawapi, Nong Khai (NK)	*T. striatus*	3	18.148606 N/103.328608 E	182	28 May 2022
2. Prangku, Sisaket (SR)	*T. striatus*	3	14.830383 N/104.060561 E	147	26 Aug 2023
	T. rubidus	3			
3. Phon Na Kaeo, Sakon Nakhon (SK2)	*T. striatus*	1	17.149833 N/104.350667 E	169	22 Oct 2023
		5			28 Nov 2023
	T. rubidus	3			28 Nov 2023
4. Ban Non Sawan, Mahasarakham (MK1)	*T. megalops*	2	16.217917 N/103.334333 E	145	2 Sep 2023
5. Na Dun, Mahasarakham (MK2)	*T. megalops*	3	15.696722 N/103.225694 E	157	5 Sep 2023
	T. striatus	2			
6. Loeng Nok Tha, Yasothon (YT)	*T. rubidus*	5	16.136194 N/104.615028 E	159	9 Sep 2023
7. Selaphum, Roi Et (RE)	*T. striatus*	5	16.181861 N/103.927278 E	145	2 Sep 2023
	T. rubidus	2			9 Sep 2023
8. Non Sang, Nong Bua Lam Phu (NP)	*T. rubidus*	3	16.805264 N/102.558381 E	190	23 Sep 2023
9. Khamriang 1, Mahasarakham (MK3)	*T. megalops*	1	16.260056 N/103.239139 E	144	29 Oct 2023
		3			11 Nov 2023
		3			
	T. striatus	3			22 Nov 2023
	T. megalops	2			27 Nov 2023
	T. striatus	2			
	T. rubidus	1			29 Oct 2023
	T. striatus	2			6 Dec 2023
	T. megalops	7			
10. Khamriang 2, Mahasarakham (MK3)	*T. rubidus*	1	16.255556 N/103.233722 E	154	5 Oct 2023
	T. megalops	1			13 Dec 2023
	T. striatus	1			
11. Khamriang 3, Mahasarakham (MK3)	*T. megalops*	3	16.253056N /103.228111 E	152	13 Dec 2023
	T. striatus	1			
	T. rubidus	1			11 Nov 2023
12. Waritchaphum, Sakon Nakhon (SK1)	*T. rubidus*	3	17.238861 N/103.573139 E	198	6 Oct 2023
13. Somdet, Kalasin (KS1)	*T. rubidus*	9	16.775222 N/103.834778 E	208	21 Oct 2023
14. Chatturat, Chaiyaphum (CP)	*T. rubidus*	10	15.650028 N/101.983547 E	191	28 Oct 2023
	T. striatus	1			
	T. megalops	4			22 Dec 2023
	T. striatus	3			
	T. striatus	1			23 Feb 2024
16. Ban Non Sabang 1, Mahasarakham (MK4)	*T. megalops*	1	16.268333 N/103.221944 E	149	11 Nov 2023
		6			23 Nov 2023
	T. striatus	5			23 Nov 2023
		2			
	T. megalops	1			27 Nov 2023
		1			29 Nov 2023
		3			13 Dec 2023
17. Ban Non Sabang 2, Mahasarakham (MK4)	*T. megalops*	1	16.267056 N/103.226250 E	151	29 Nov 2023
		2			13 Dec 2023
18. Chiang Yuen, Mahasarakham (MK5)	*T. megalops*	3	16.379167 N/103.054333 E	164	31 Aug 2023
19. Chaloem Phra Kiat, Nakhon Ratchasima (NS1)	*T. rubidus*	3	14.924818 N/102.252981 E	197	10 Aug 2024
20. Mueang Nakhon Ratchasima (NS2)	*T. rubidus*	2	14.942953 N/102.225903 E	177	10 Aug 2024
21. Huai Phueng, Kalasin (KS2)	*T. striatus*	4	16.754056 N/103.850306 E	210	9 Dec 2023

antennae, eyes, frons, proboscis, body, legs, and wing coloration and patterns.

Molecular study

DNA was extracted from head and thorax from each individual specimen using the GF-1 Nucleic Acid DNA extraction kit (Vivantis Technologies Sdn. Bhd., Malaysia). Approximately 650-bp of the mitochondrial cytochrome oxidase I (COI) gene was amplified using the primers LCO1490 (5’-GGTCAACAAATCATAAAGATATTGG-3’) and HCO2198 (5’-TAAACTTCAG GGTGACAAAAAATCA-3’) (Folmer *et al*., 1994). The PCR reaction conditions followed those of Tangkawanit *et al*. (2018) using TaKaRa Ex Taq™ (TaKaRa Bio, Shiga, Japan). PCR products were stained with Novel Juice (GeneDirex, Taoyuan, Taiwan, Republic of China) and were checked using 1% agarose gel electrophoresis. Successful amplifications were purified using the PureDireX PCR CleanUp & Gel Extraction kit (Bio-Helix, Taiwan, Republic of China). Purified PCR products were sequenced in both directions at ATCG Company Limited (Thailand Science Park, Pathumthani, Thailand) using the same primers as for PCR.

Data analysis

In total, 137 specimens (47 for *T. megalops*, 46 for *T. rubidus* and 44 for *T. striatus*) were obtained for molecular study. The COI sequences of these specimens were deposited in NCBI GenBank under accession numbers PV133567 – PV133703. Three genetic diversity indices were calculated: p-distance, haplotype diversity and nucleotide diversity. The p-distance was estimated in TaxonDNA (Meier *et al*., 2006) and haplotype and nucleotide diversity were calculated using Arlequin ver. 3.5 (Excoffier & Lischer, 2010). Within species genetic relationships were inferred using median joining (MJ) networks (Bandelt *et al*., 1999) using NETWORK ver. 10.2.0.0 software (https://www.fluxus-engineering.com) (accessed on 17 January 2024). To examine phylogenetic relationships between COI sequences of *Tabanus* species from the present study with existing data recorded in the public database, conspecific sequences available in the Barcode of Life Data System (https://boldsystems.org/) (accessed on 2 January 2025) were also retrieved and included in the data analyses. Two phylogenetic methods, neighbor joining (NJ) and maximum likelihood (ML) were used to infer genetic relationships between these *Tabanus* species. Both NJ and ML analyses were performed using MEGA X (Kumar *et al*., 2018). Branch support was calculated based on 1,000 bootstrapping replications. The COI sequences of *T. striatus* (accession nos. KM111709 and KM111713) were used as outgroups for the phylogenetic analyses of *T. rubidus*. For the phylogenetic analyses of *T. striatus* and *T. megalops*, COI sequences of *T. rubidus* (accession nos. MG426142–3) were used as the outgroups. A species delimitation method (Assemble Species by Automatic Partitioning (ASAP; Puillandre *et al*., 2021), was used to determine cryptic diversity within each species. ASAP assigned species using the magnitude of genetic distances within and between defined partitions (delimited species) (Puillandre *et al*., 2021). The ASAP analysis was conducted using the web server version (available at https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) (accessed on 18 January 2025) based on default mode with a K80 substitution model. Because *T. striatus* and *T. megalops* are closely related species belonging to the same complex, we therefore combined data of these species for MJ network, NJ and ML phylogenetic analyses.

RESULTS

Genetic diversity of *Tabanus rubidus*

There were 41 polymorphic sites detected within a 654-bp fragment of the COI gene sequences. Intraspecific genetic divergence based on uncorrected p-distance among specimens varied from 0% to 2.14%. Included sequences of this species recorded in the BOLD database (n = 125) revealed much higher intraspecific genetic divergence values varying from 0% to 4.43%. The greatest genetic divergence was between a specimen from India and one from Thailand. Despite finding low intraspecific genetic divergence within Thai specimens collected in this study, the haplotype diversity was high with 33 haplotypes identified from 46 specimens. The overall haplotype diversity was 0.9778 and varied between 0.8667 and 1.0000. The nucleotide diversity varied from 0.0031 to 0.0143 with an overall value of 0.0097 (Table 2).

Table 2. Genetic diversity indices in each sampling location of three *Tabanus* species from northeastern Thailand. Details of each sampling site are provided in Table 1

Species/ Location	N	No. of haplotypes	Haplotype diversity (±S.E.)	Nucleotide diversity (±S.E.)
T. megalops				
MK1	2	2	1.0000 ± 0.5000	0.0031 ± 0.0038
MK2	3	3	1.0000 ± 0.2722	0.0272 ± 0.0210
MK3	20	10	0.8211 ± 0.0746	0.0138 ± 0.0074
MK4	15	12	0.9714 ± 0.0327	0.0185 ± 0.0099
MK5	3	2	0.6667 ± 0.3143	0.0010 ± 0.0013
CP	4	3	0.8333 ± 0.2224	0.0197 ± 0.0135
Total for *T. megalops*	47	26	0.8982 ± 0.0361	0.0163 ± 0.0084
T. rubidus				
KS1	9	8	0.9722 ± 0.0640	0.0094 ± 0.0056
CP	10	7	0.8667 ± 0.1072	0.0098 ± 0.0057
SR	3	3	1.0000 ± 0.2722	0.0124 ± 0.0099
YT	5	5	1.0000 ± 0.1265	0.0143 ± 0.0093
RE1	2	2	1.0000 ± 0.5000	0.0031 ± 0.0038
NP	3	3	1.0000 ± 0.2722	0.0113 ± 0.0091
MK3	3	3	1.0000 ± 0.2722	0.0114 ± 0.0091
SK1	3	3	1.0000 ± 0.2722	0.0093 ± 0.0075
SK2	3	3	1.0000 ± 0.2722	0.0093 ± 0.0076
NS1	3	3	1.0000 ± 0.2722	0.0051 ± 0.0045
NS2	2	2	1.0000 ± 0.5000	0.0031 ± 0.0038
Total for *T. rubidus*	46	33	0.9778 ± 0.0109	0.0097 ± 0.0052
T. striatus				
NK	3	2	0.6667 ± 0.3143	0.0062 ± 0.0052
SR	3	3	1.0000 ± 0.2722	0.0041 ± 0.0037
SK2	6	6	1.0000 ± 0.0962	0.0064 ± 0.0042
MK2	2	2	1.0000 ± 0.5000	0.0234 ± 0.0241
MK3	9	8	0.9778 ± 0.0540	0.0189 ± 0.0106
MK4	7	7	1.0000 ± 0.0764	0.0062 ± 0.0040
KS2	4	4	1.0000 ± 0.1768	0.0049 ± 0.0038
CP	5	4	0.9000 ± 0.1610	0.0025 ± 0.0020
RE	5	4	0.9000 ± 0.1610	0.0025 ± 0.0020
Total for *T. striatus*	44	36	0.9831 ± 0.0111	0.0125 ± 0.0066

The MJ network revealed no divergent lineages or geographic associations of the haplotypes (Figure 2). The NJ and ML trees exhibited similar tree topologies, therefore, only the latter is presented (Figure 3). The ML tree revealed three genetically divergent clades (I, II, and III). All Thai specimens obtained in the present study and those retrieved from BOLD database clustered together along with specimens from Bangladesh and Malaysia (each represented by only a single specimen), to form clade I. This clade corresponded to the BIN (Barcode Index Number, a group of sequences representing presumptive species automatically generated in BOLD) BOLD:ACV0798 identified in BOLD. Clade II comprised specimens from Bangladesh, India and Pakistan and represented the BIN BOLD:AAZ4274. A single specimen from India represented clade III and was resolved as BIN BOLD:AAU6718 in BOLD. The ASAP species delimitation determined that there were three possible species within the *T. rubidus* corresponding with the three clades of the ML tree.

Genetic diversity of *Tabanus megalops* and *T. striatus*

A total of 47 specimens of *T. megalops* collected from six locations were examined in this study. There are 45 polymorphic sites within 654-bp sequences of the COI gene of this species. Intraspecific genetic divergence varied between 0% and 4.43%. Included COI sequences of this species available in BOLD were all from Thailand, and had slightly increased maximum intraspecific genetic divergence at 4.59%. The overall nucleotide diversity was 0.0163 and varied between 0.0010 and 0.0197 across sampling locations. The overall haplotype diversity was 0.8982 and varied from 0.6667 to 1.0000 in each sampling location (Table 2).

A total of 44 specimens of *T. striatus* collected from eight locations were included in this study. In total, 50 polymorphic sites were detected in the 654-bp sequences of the COI gene. High intraspecific genetic divergence was found within these specimens with a maximum value of 5.20%. Included COI sequences available in BOLD (62 sequences) had slightly increased maximum intraspecific genetic divergence at 5.81%. Greatest genetic divergence was found between specimens in the present study compared to those recorded from India. The overall haplotype diversity of the Thai specimens from this study was 0.9831 and varied from 0.6667 to 1.0000 in each sampling location. Overall nucleotide diversity was 0.0125, varying from 0.0025 to 0.0234 across sampling sites (Table 2).

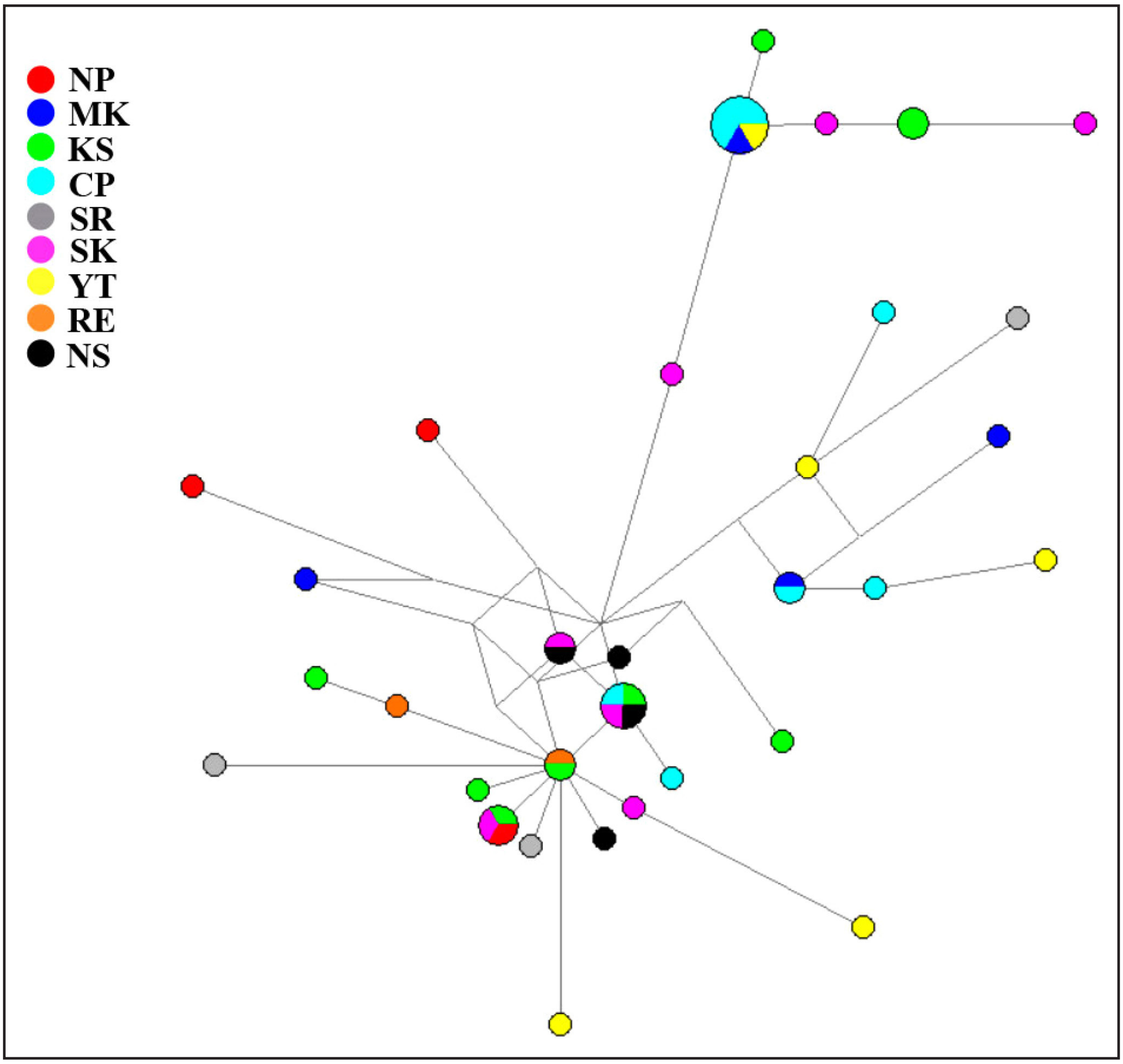

Figure 2. Median joining network of 46 COI sequences of *Tabanus rubidus* collected from northeastern Thailand. Each circle represents the COI haplotype. The sizes of circles is relative to the number of individuals sharing such a haplotype. Colors of the haplotypes indicate sampling location by province (NP, Nong Bua Lam Phu; MK, Mahasarakham; KS, Kalasin; CP, Chaiyaphum; SR, Sisaket; SK, Sakon Nakhon; YT, Yasothon; RE, Roi Et; NS, Nakhon Ratchasima). Details of each sampling site are provided in Table 1.

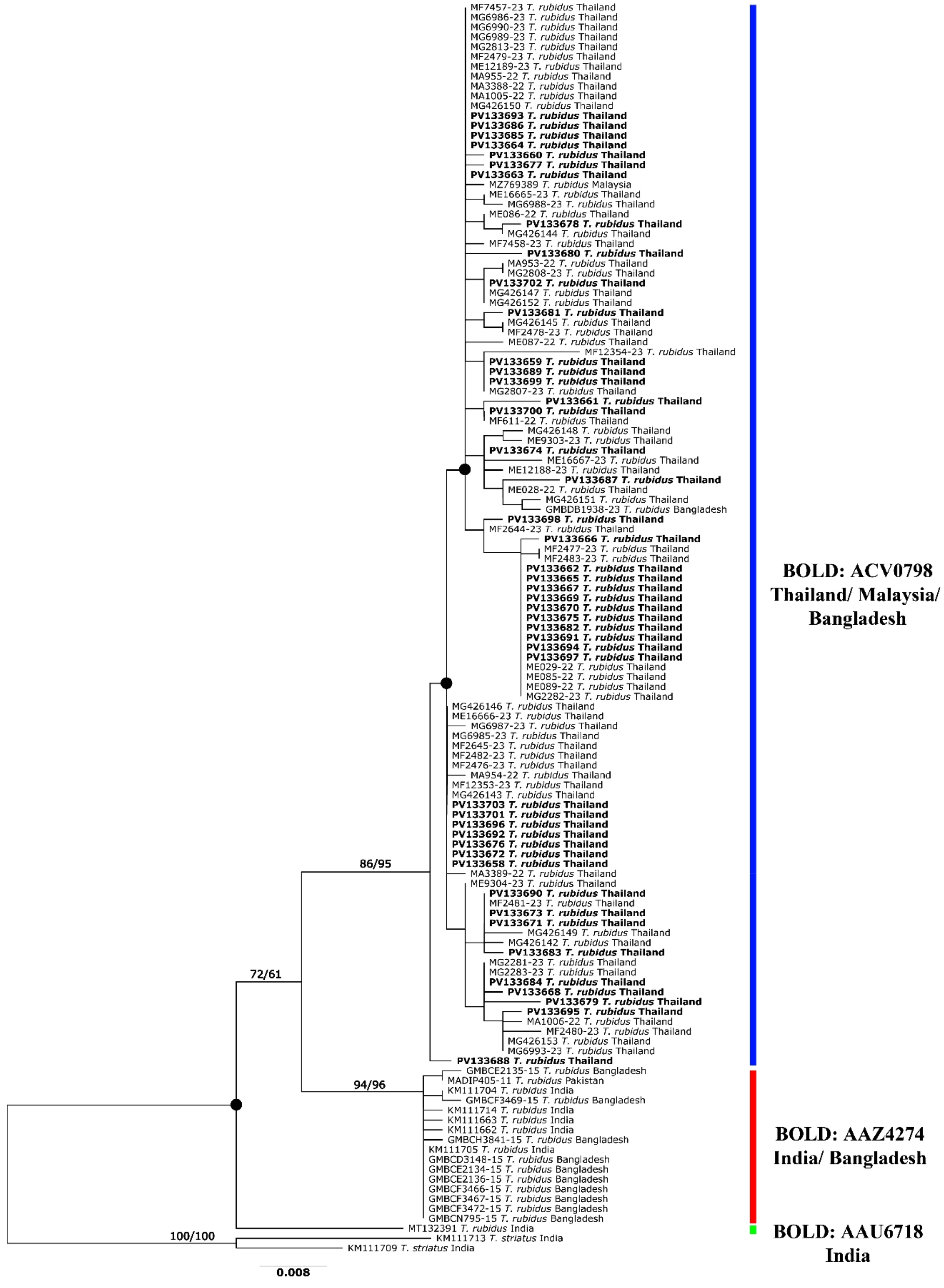

Figure 3. Maximum likelihood tree of *Tabanus rubidus* based on the mitochondrial cytochrome I sequences. Bootstrap values based on the ML and NJ analyses are shown above or near the branch. The filled circles at internal nodes indicate the clade has low (<50% support). Bold indicate specimens obtained in the present study. The GenBank accession number or BOLD specimen ID, species name and country of origins are provided for each specimen. Vertical bars indicate species delimited based on the ASAP method. Each delimited species is indicated by different colors.

The MJ network revealed three genetically divergent lineages within *T. striatus* and *T. megalops* (Figure 4). Lineage I comprised specimens of both species while lineages II and III exclusively represented *T. megalops* and *T. striatus*, respectively. These genetic lineages of *T. striatus* and *T. megalops* were not associated with geographic origins (i.e. collection province) (Figure 5). Specimens collected across geographically distant provinces belonged to the same lineage. Lineage I was comprised of specimens of both species which had been collected from all provinces with greatest distance between sampling locations of >370 km (between SR and NK). Similarly, lineage II comprised specimens of *T. megalops* from two provinces, Mahasarakham (MK) and Chaiyaphum (CP) geographically separated by a distance of approximately 150 km. Lineage II comprised specimens from MK and Kalasin (KS) with >80 km geographic isolation.

Phylogenetic analyses based on NJ and ML methods revealed similar tree topologies, therefore, only the ML tree is presented here (Figure 6). The ML tree included three main clades, one of which

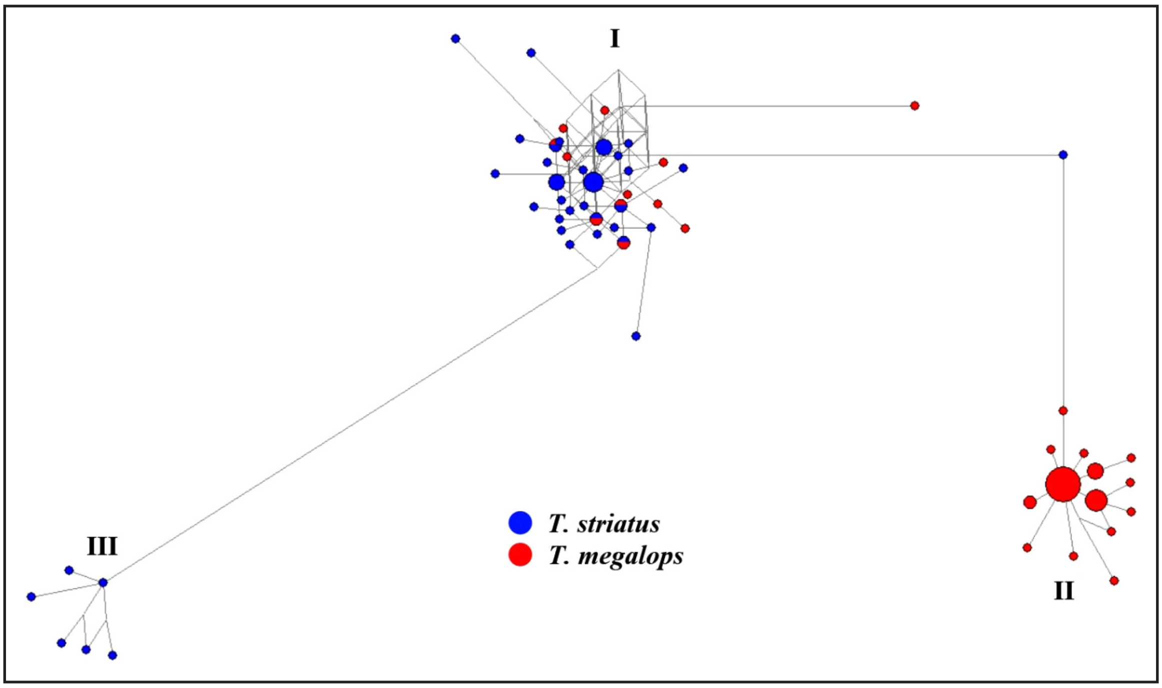

Figure 4. Median joining network of 91 COI sequences of *Tabanus megalops* (n = 47) and *T. striatus* (n = 44) collected from northeastern Thailand. Each circle represents the COI haplotype. The sizes of circles are relative to the number of individuals sharing such a haplotype. Colors of the haplotype indicate species.

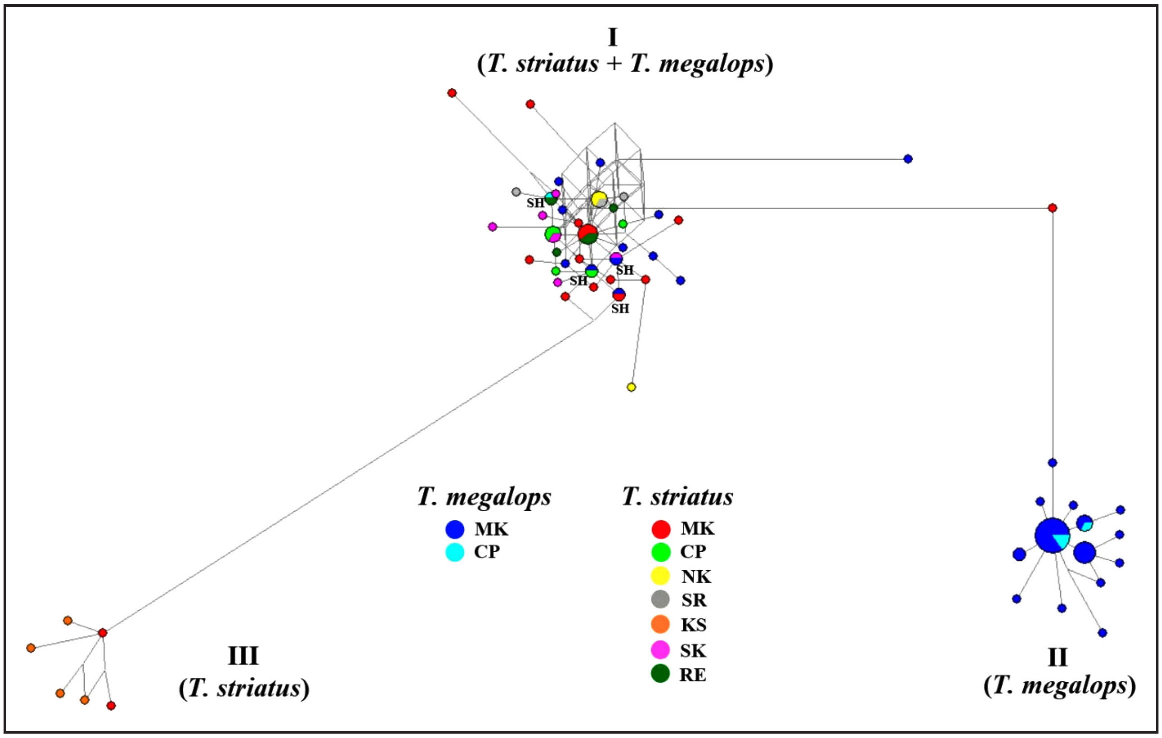

Figure 5. Median joining network of 91 COI sequences of *Tabanus megalops* (n = 47) and *T. striatus* (n = 44) collected from northeastern Thailand. Each circle represents the COI haplotype. Size of the circle is relative to the number of individuals sharing such a haplotype. Colors of the haplotype indicate sampling location and species (MK, Mahasarakham; KS, Kalasin; CP, Chaiyaphum; SR, Sisaket; SK, Sakon Nakhon; RE, Roi Et; NK, Nong Khai). Details of each sampling site are provided in Table 1. SH indicates haplotype sharing between *T. megalops* and *T. striatus*.

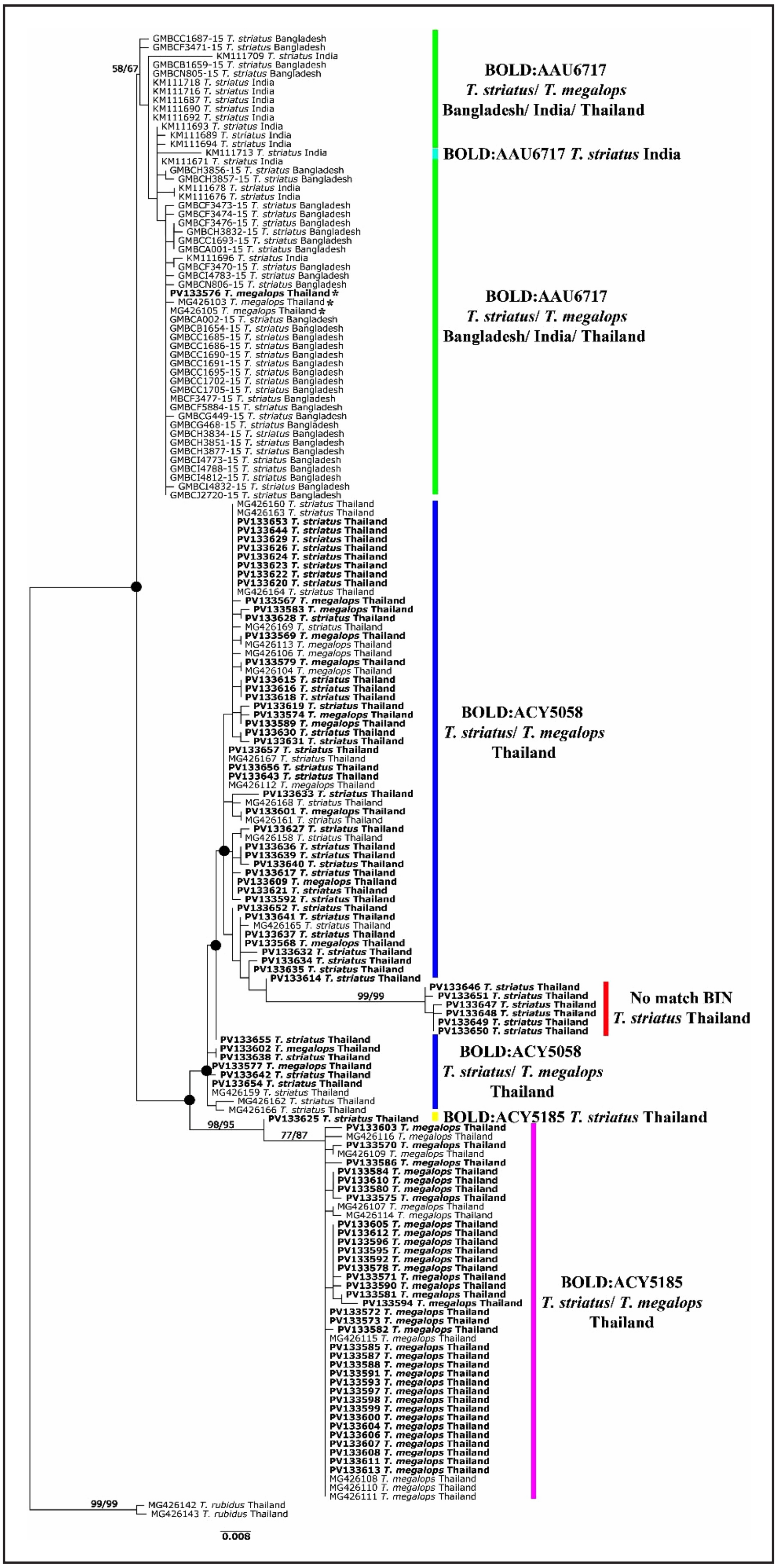

Figure 6. Maximum likelihood tree of *Tabanus striatus* and *T. megalops* based on the mitochondrial cytochrome I sequences. Bootstrap values based on the ML and NJ analyses are shown above or near the branch. The filled circles at internal nodes indicate the clade has low support (<50%). Bold indicate specimens obtained in the present study. The GenBank accession number or BOLD specimen ID, species name and country of origins are provided for each specimen. Vertical bars indicate species delimited based on ASAP method. Each delimited species is indicated by different colors. Asterisks indicate specimens possibly misidentificatied.

comprised the majority of *T. striatus* from Bangladesh and India. The exception was three sequences from Thailand, one from the present study and the other two from those recorded in GenBank that identified as *T. megalops* were also included in the clade. This clade represented members of the BIN BOLD:AAU6717 identified in BOLD. The second clade comprised both *T. striatus* and *T. megalops* and it was exclusively representative only of specimens originating from Thailand. The members of this clade were identified to the BIN BOLD:ACY5058. However, six specimens from the present study included in this clade were genetically distinct from others and all were identified as *T. striatus*. The third clade, like the second, comprised both *T. striatus* and *T. megalops* and all were from Thailand. This clade represented BIN BOLD:ACY5185.

The ASAP analysis identified six species within *T. striatus* + *T. megalops* (Figure 6). All three BINs were treated as different species. However, specimens of *T. striatus* from India recorded in GenBank (accession no. KM111713) and one from the present study (accession no. PV133625) were each was treated as different species. Six specimens of *T. striatus* from the present study that formed a divergent lineage were also treated as different species.

DISCUSSION

Intraspecific genetic divergence in the northeastern region of Thailand (2.14%) found in this study was slightly higher than in a previous report for the specimens obtained from other regions of the country (max. = 1.9%). However, the level of genetic diversity for *T. rubidus* from Thailand was much higher than in those from India (max. = 0.87%). This differentiation was possibly influenced, in part, by the number of specimens examined where only five individuals from a single location were investigated in India (Banerjee *et al*., 2015) and 12 individuals from a previous study in Thailand (Changbunjong *et al*., 2018b) were compared to 46 individuals in the present study. Despite possessing a relatively high genetic diversity, there was no indication of genetic structuring of *T. rubidus* within Thailand as revealed by haplotypes from each sampling site being distributed across the MJ network. No genetic structuring could be related to dispersal ability of the horse flies as they are capable of dispersing over several kilometers (Mullens, 2019).

Phylogenetic analysis indicated that specimens of *T. rubidus* collected in the present study and those previously reported from Thailand were genetically distinct from those of India, Pakistan and Bangladesh with a minimum genetic distance of 1.99%. There are two possible explanations for the existence of genetically divergent lineages within *T. rubidus*: 1) they represent the same species but with genetic structuring due to geographic isolation or 2) the genetic lineages represent different but morphologically cryptic species. Although the first explanation is also possible the evidence suggests that the second is more likely to explain the existence of the two genetic lineages. The level of genetic distance between these lineages was higher than those of interspecific genetic divergent of closely related Tabanid species. Cywinska *et al*. (2010) found that the interspecific genetic divergence between congeneric species of a Canadian Tabanid was 1.5%. Similarly, Changbunjong *et al*. (2018b) found that some closely related *Tabanus* species in Thailand had no (0%) or low (1.7%) genetic differentiation. Both lineages were treated as different species by BIN and ASAP analyses. These lineages are geographically isolated, occurring in Southeast Asia (Thailand and Malaysia) and South Asia (India, Pakistan and Bangladesh). The exception was one specimen from Bangladesh which was placed in the Southeast Asia clade. Geographical coexistence of genetically divergent lineages indicates the possibility of reproductive isolation (Hausdorf & Hennig, 2020). The type locality of *T. rubidus* is presumably from India (Burton, 1978). Therefore, specimens from India and those belonging to the same genetic lineage are more likely to be true *T. rubidus* while the Thailand + Malaysia lineage is possibly a different previously unrecognized species. Further study using additional genetic markers and in-depth morphological examination will be helpful to test this hypothesis.

Tabanus megalops and *T. striatus* are members of the *T. striatus*-complex (Burton, 1978) that were examined in this study. Three distinct genetic lineages were found in the specimens from the northeastern region of Thailand. Two were species specific, one for *T. megalops* and one for *T. striatus* and another which included the majority of the specimens comprised both species. Because both species were comprised of specimens that belong to different genetic divergent lineages, the corresponding intraspecific genetic divergence values were high (max. 4.43% and 5.20% for *T. megalops* and *T. striatus*, respectively). A close genetic relation to other species than the conspecific might result from mitochondrial introgression (Funk & Omland, 2003). These two species are closely related and geographically coexist in many areas in Thailand (Burton, 1978; Changbunjong *et al*., 2018a). Therefore, there is a possibility of interspecific hybridization has occurred. Interspecific hybridization events can be indicated by haplotype sharing among sympatric populations of genetically distinct species (Funk & Omland, 2003). We found that four haplotypes were shared between *T. megalops* and *T. striatus* and only one of these was from the same locality (MK) while the others were from geographically isolated populations. Therefore, the possibility of mitochondrial introgression as a result of interspecific hybridization is low. Further study using nuclear genetic markers could test the hypothesis of mitochondrial introgression as a result of interspecific hybridization.

Other possible explanations are incomplete lineage sorting, species misidentification and imperfect taxonomy (Funk & Omland, 2003). *Tabanus megalops* and *T. striatus* are morphologically assigned to the *T. striatus* complex and hence they are closely related species (Burton, 1978). The incomplete lineage sorting because of the recent divergence can explain sharing haplotypes between species. Three specimens from Thailand identified as *T. megalops* were placed in the *T. striatus* clade are likely to be species misidentifications. These two species are morphologically very similar and therefore, morphological identification can be problematic (Burton, 1978). A DNA barcoding study from Thailand found that these species cannot be distinguished based on their COI gene sequences because some specimens morphologically identified as *T. melalops* are genetically closer to the *T. striatus* than the conspecific (Changbunjong *et al*., 2018b). However, wing geometric morphometrics can be effectively used for identification of these species with an accuracy of 86% and 96% based on wing cell contour (Changbunjong *et al*., 2024) and wing landmarks (Changbunjong *et al*., 2021) respectively.

The taxonomy of the *T. striatus*-complex is clearly imperfect which uncertainties arising from the existence of an additional genetically divergent lineage representative of specimens of both *T. striatus* and *T. megalops* from Thailand (BOLD: ACY508). These lineages have a considerable level of genetic divergence (1.38%) although within the range of intraspecific genetic divergence for Tabanidae (Cywinska *et al*., 2010; Banerjee *et al*., 2015; Changbunjong *et al*., 2018b). Therefore, these lineages possibly represent within-species genetic structure. However, the lineage contained individuals collected from geographically different locations that were genetically distinct from sympatric populations and therefore indicate the possibility of reproductive isolation (Hausdorf & Hennig, 2020). This also supported by BIN and ASAP analyses as both species delimitation methods treated this lineage as different species. Further study using additional genetic markers from nuclear DNA will be helpful to assure that these genetically divergent lineages do indeed represent the same species or that they are different but morphologically similar species.

In conclusion, we found relatively high level of genetic diversity within three common horse fly species, *T. rubidus*, *T. striatus* and *T. megalops*. This is in contrast to previous barcoding studies (Banerjee *et al*., 2015; Changbunjong *et al*., 2018a) where relatively low diversity was recorded. The discrepancies are potentially due to different sample sizes (<12 vs >41 per species) and highlight the necessity of using an adequate number of specimens from each different geographic location so as to fully account for intraspecific variation (Meyer & Paulay, 2005; Phillips *et al*., 2019). A high level of genetic diversity is due to the existence of cryptic species within examined specimens. Two and three genetically divergent lineages in *T. rubidus* and *T. striatus* + *T. megalops* were found, respectively. Further study using additional genetic markers particularly those from nuclear DNA will be helpful to test for species status within this cryptic diversity. Solving the taxonomic status of genetic divergent lineages within vector species is particularly important because it can be related to pathogen competency. Despite considerable genetic diversity, there is no indication of genetic structuring within the northeastern region of Thailand. Furthermore, comparison with specimens from other geographic regions of Thailand reported previously (Changbunjong *et al*., 2018b) also found no signal of genetic structuring. Genetic homogeneity of these horse fly vector species indicate that they can transmit the pathogens across a large geographic scale. Therefore, control and prevention program should be focus on country-wide strategy.

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Conflict of Interest

The author declares that they have no conflict of interests.

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