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RESEARCH ARTICLE

Common but not connected: high genetic structure and cryptic genetic diversity in the ubiquitous biting midge *Culicoides peregrinus* Kieffer

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ABSTRACT

The biting midge *Culicoides peregrinus* Kieffer is a significant pest and vector species, and knowledge of its genetic diversity and genetic structure is critically important for designing an effective control program. However, such information is limited to only small sample-size DNA barcoding studies. Therefore, in this study, we used mitochondrial cytochrome *c* oxidase I (COI) to examine genetic structure and diversity of *C. peregrinus* from northeastern Thailand. In addition, we also inferred genetic relationships between *C. peregrinus* from Thailand and those reported from other countries across the geographic range of the species. Maximum intraspecific genetic divergence (3.83%) within Thai specimens was relatively high compared to other *Culicoides* species. Genetic structure analysis revealed that 71% (32 from 45) of population comparisons were highly significantly different. A high level of genetic structure among populations, even between those in close geographic proximity (22 km geographic distance) suggested that there has been little or no movement between local populations. This is possibly due to the ability to exploit diverse types of breeding site and a generalist feeding habit which enables *C. peregrinus* to complete its life cycle within cattle pens. Genetic relationships between Thai *C. peregrinus* and those reported from other countries revealed three genetically divergent lineages (A, B and C) associated with geographic origins. Specimens from Thailand + China formed lineage A, those from Australia formed lineage B and India + Bangladesh belonged to lineage C. These genetically divergent lineages also agree with morphological variation of the wing pale marking spots. Further investigation using independent genetic loci from nuclear genes will be very useful to resolve taxonomic status of these divergent lineages.

Keywords: Biting midge; DNA barcode; genetic diversity; genetic structure; population genetics.

INTRODUCTION

Biting midges of the genus *Culicoides* Latreille (Diptera: Ceratopogonidae) are small (<2.5 mm in body length) blood-sucking insects. There are >1,300 extant species recorded globally (Borkent & Dominiak, 2020). Many of these are vectors of pathogens that can be transmitted to humans and other animals, causing diseases such as Oropouche fever in humans, bluetongue disease in ruminants, African horse sickness in equines, and leucocytozoonosis in chickens (Mullen & Murphree, 2019).

Culicoides peregrinus Kieffer is a member of the subgenus *Hoffmania* Fox of the genus *Culicoides* Latreille. This species was distinguished as a potent vector of the bluetongue virus (BTV) in Australia (Mullen & Murphree, 2019) and India (Ranjan *et al*., 2017). *Culicoides peregrinus* was described from the Orrisa Coast, India (Wirth & Hubert, 1989). The species is geographically widespread being recorded from India, Sri Lanka, Bangladesh, Southeast Asia, Australia, Papua New Guinea, Japan and Southern China (Sen & Gupta, 1959; Wirth & Hubert, 1989; Dyce *et al*., 2007; Liu *et al*., 2018). *Culicoides peregrinus* is a common species often found in high abundance in or near the animal farms (Harsha *et al*., 2020; Fujisawa *et al*., 2021; Kar *et al*., 2022a). This species has exploited a wide variety of breeding sites including shaded pools and pond margins, drains and stream margins, paddy fields as well as organic rich substrates (Wirth & Hubert, 1989; Yanase *et al*., 2013; Harsha & Mazuda, 2015). Based on the biting habit, *C. peregrinus* was considered as a general feeder because it feeds on diverse hosts including humans, horse, goat, sheep, cow, buffalo, pig, dog and birds (Wirth & Hubert, 1989; Jomkumsing *et al*., 2021; Kar *et al*., 2022b; Sunantaraporn *et al*., 2022; Kamyingkird *et al*., 2023).

Despite its significance as a pest and potent vector, little is known about the genetic diversity and genetic structure of *C. peregrinus* except for DNA barcoding studies based on small sample sizes (Harrup *et al*., 2016; Liu *et al*., 2018; Jomkumsing *et al*., 2021; Gopurenko *et al*., 2022). Understanding the genetic diversity and genetic structure of the vector species is important for disease epidemiology and might be used to design an effective control program (Tabachnick & Black, 1995; McCoy, 2008). For example, the level of genetic differentiation could indicate rate of individual movement between local populations. This information is critically important for vector control strategy (Tabachnick & Black, 1995). For species that are widely distributed, such as those found across different biogeographic regions, it is also important to test species status between geographically distant populations as it could be

Table 1. Sampling locations, number of specimen (N), number of haplotypes, nucleotide diversity (π) and haplotype diversity (*h*) of *Culicoides peregrinus* from Thailand

Location	Latitude/Longitude	N (no. of haplotypes)	π±S.E.	*h*±S.E	Collection date
1. Prangku, Si Sa Ket (SR)	14.83035 N/104.06043 E	13 (8)	0.0085±0.0050	0.8077±0.1131	2 Jan 2021/ 4 Jun 2022
2. Huai Mek, Kalasin (KS1)	16.60528 N/ 103.22320 E	18 (6)	0.0019±0.0014	0.5621±0.1342	23 Jan 2021
3. Nadun, Maha Sarakham (MK1)	15.68681 N/ 103.23162 E	9 (9)	0.0066±0.0041	1.0000±0.0524	20 Feb 2021
4. Ban Don Suan, Kantharawichai, Maha Sarakham (MK2)	16.25278 N/ 103.27525 E	12 (8)	0.0098±0.0057	0.9091±0.0649	26 Feb 2021
5. Non Sang, Nong Bua Lamphu (NL)	16.82201 N/ 102.57699 E	6 (6)	0.0047±0.0034	1.0000±0.0962	27 Feb 2021
6. Ban Hua Na Kam, Kalasin (KS2)	16.40762 N/ 103.20411 E	18 (10)	0.0063±0.0037	0.8497±0.0775	19 Nov 2020
7. Ban Pho Si, Nakhon Phanom (NP)	17.44680 N/ 104.46520 E	10 (9)	0.0200±0.011	0.9778±0.0540	20 Nov 2020
8. Khongchiam, Ubon Ratchathani (UB)	15.31459 N/ 105.49841 E	16 (13)	0.0084±0.0048	0.9667±0.0357	12 Nov 2022
9. Ban Nong Hua Mu, Udon Thani (UD)	17.36152 N/ 102.75725 E	10 (7)	0.0068±0.0042	0.9111±0.0773	13 Oct 2022
10. Trang (TR)[a]	7.83065 N/ 99.33763 E [a]	3 (1)[a]	0	0	29 Sep 2012 [a]
Total		**115 (64)**	**0.0082±0.0044**	**0.9466±0.0138**	

[a]Data from Gopurenko *et al*. (2022).

related to the vector-pathogen competency (McCoy, 2008; Powell, 2018). Morphological identification of *Culicoides* is difficult due to their small size and low within-species morphological variation which provide only limited diagnostic characters distinguishing closely related species (Harrup *et al*., 2015). Therefore, genetic markers such as DNA barcodes can be very helpful for testing species status (Ander *et al*., 2013; Harrup *et al*., 2016). An example of this type of study is the discovery of morphologically similar but genetically different species within the so-called *C. arakawae* (Arakawa) in Thailand (Pramual *et al*., 2021b). *Culicoides arakawae* is geographically widely distributed from southern, eastern and southeastern Asia and Japan (Wirth & Hubert, 1989). DNA barcode analyses revealed that some specimens formerly identified as *C. arakawae* are actually different species which were then formally described as *C. mahasarakhamense* (Pramual *et al*., 2021b). Recent studies have revealed that *C. mahasarakhamense* is a potent vector of several disease-causing agents including *Leucocytozoon*, *Plasmodium juxtanucleare*, *P. gallinaceum* and *Leishmania martiniquensis* (Pramual *et al*., 2021a; Sunantaraporn *et al*., 2021; Songumpai *et al*., 2022).

In this study, we examined the genetic diversity and genetic structure based on the mitochondrial cytochrome *c* oxidase I (COI) gene sequences of *C. peregrinus* collected from cattle pens in northeastern Thailand. By using a population genetic approach, the level of genetic differentiation between local populations can be inferred. This information can then be used to indicate inter-population movement which can potentially be used for designing effective control programs (Tabachick & Black, 1995). We also examined genetic relationships between specimens of *C. peregrinus* from Thailand with those reported from other countries. This enabled determination of species status of populations from geographically widespread regions.

MATERIALS AND METHODS

Specimen collection and identification

Adult fly specimens of *C. peregrinus* were collected around cattle pens from nine locations (Table 1 and Figure 1) in northeastern Thailand between 19 November 2020 and 12 November 2022. Specimens were collected by a sweep net swept around the animal host and in the air around the cattle pens. Specimens were preserved in 80% ethanol and stored at -20°C in a freezer until use. The species was identified using keys and descriptions of Southeast Asian biting

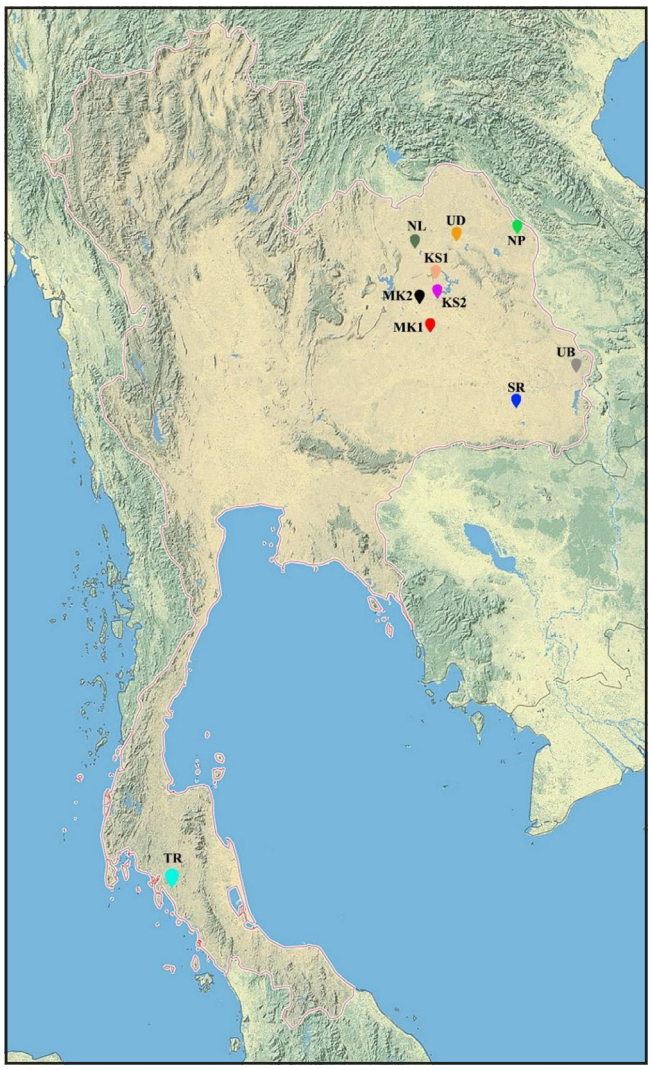

Figure 1. Map of Thailand (obtained from Food and Agriculture Organization of the United Nations available at https://www.fao.org/faostat/en/#country/216) indicating the 9 sampling locations of *Culicoides peregrinus* obtained in this study plus one (TR) retrieved information from Gopurenko *et al*. (2022). Details of sampling locations are included in Table 1.

midge (Wirth & Hubert, 1989) and wing pictorial illustration of Dyce *et al*. (2007).

DNA extraction, polymerase chain reaction (PCR), and sequencing. DNA was extracted from individual flies using the GF-1 Nucleic Acid Extraction Kit (Vivantis Technologies Sdn. Bhd, Malaysia). A fragment of approximately 650 bp of mitochondrial cytochrome *c* oxidase I (COI) was amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al*., 1994). PCR reaction conditions followed those described in Tangkawanit *et al*. (2018). 1% agarose gel electrophoresis was used to check the PCR products. Successful amplifications were purified using PureDireX PCR CleanUp & Gel Extraction Kit (BioHelix, Taiwan). Purified PCR products were sent for sequencing at the ATCG Company Limited (Thailand Science Park (TSP), Pathumthani, Thailand) using the same primers as for PCR.

Data analysis

A total of 112 COI sequences (GenBank accession nos. OR123081 - OR123192) were obtained in the present study. The COI sequences of *C. peregrinus* publicly available in NCBI GenBank and BOLDs (79 sequences) were retrieved and included in data analysis. Intraspecific genetic divergence was calculated using the uncorrected p-distance in TaxonDNA (Meier *et al*., 2006). Haplotype diversity and nucleotide diversity were estimated in ARLEQUIN ver. 3.5 (Excoffier & Lischer, 2010). Genetic relationships between haplotypes were inferred in the software Network ver. 10.2.0.0 (https://www.fluxus-engineering.com) using the median-joining (MJ) network method (Bandelt *et al*., 1999). To test the genetic differentiation between populations, pairwise F_{ST} values were calculated in ARLEQUIN. Tests of statistical significance of the F_{ST} values were based on 1023 permutations. To avoid type I error due to multiple comparisons, the Bonferroni correction was used to adjust the significance level. To examine relationships between level of genetic (based on F_{ST} values) and geographic distances (km) between sampling locations, the isolation-by-distance (IBD) model (Mantel, 1967) was tested. The IBD analysis was performed in IBD ver. 1.52 (Bohonak, 2002). Statistical significance of the IBD was based on 1,000 randomizations.

RESULTS AND DISCUSSION

Nucleotide diversity in populations ranged from 0 to 0.0098 with an overall value of 0.0082. The haplotype diversity within populations ranged between 0 and 1 with an overall value of 0.9466 (Table 1). Instraspecific genetic divergence across individual specimens (n = 112) obtained in the present study ranged between 0 – 3.14%. When COI sequences of *C. peregrinus* (n = 34) from Thailand previously reported (Jomkumsing *et al*., 2021; Gopurenko *et al*., 2022) were included, there was a slight increase of maximum intraspecific genetic divergence to 3.83%. It has been suggested that, for *Culicoides* species, the p-distance is usually <3% (Gopurenko *et al*., 2015). Species with >3% based on COI sequences are likely to be comprised of cryptic species, if there is also support from other evidence such as additional genetic markers or morphological characteristics or with a highly genetically different lineage at an early stage of speciation. Based on the MJ network (Figure 2) of the Thai specimens, there were no indications of deep genetic divergence of lineages except for three genetically divergent haplotypes. If these haplotypes were omitted, maximum genetic divergence within species of *C. peregrinus* in Thailand was only 2.44%. This level of intraspecific genetic divergence is similar to that reported for *C. peregrinus* from India (2.0%) (Harrup *et al*., 2016) although our study had much larger sample size (146 vs 10).

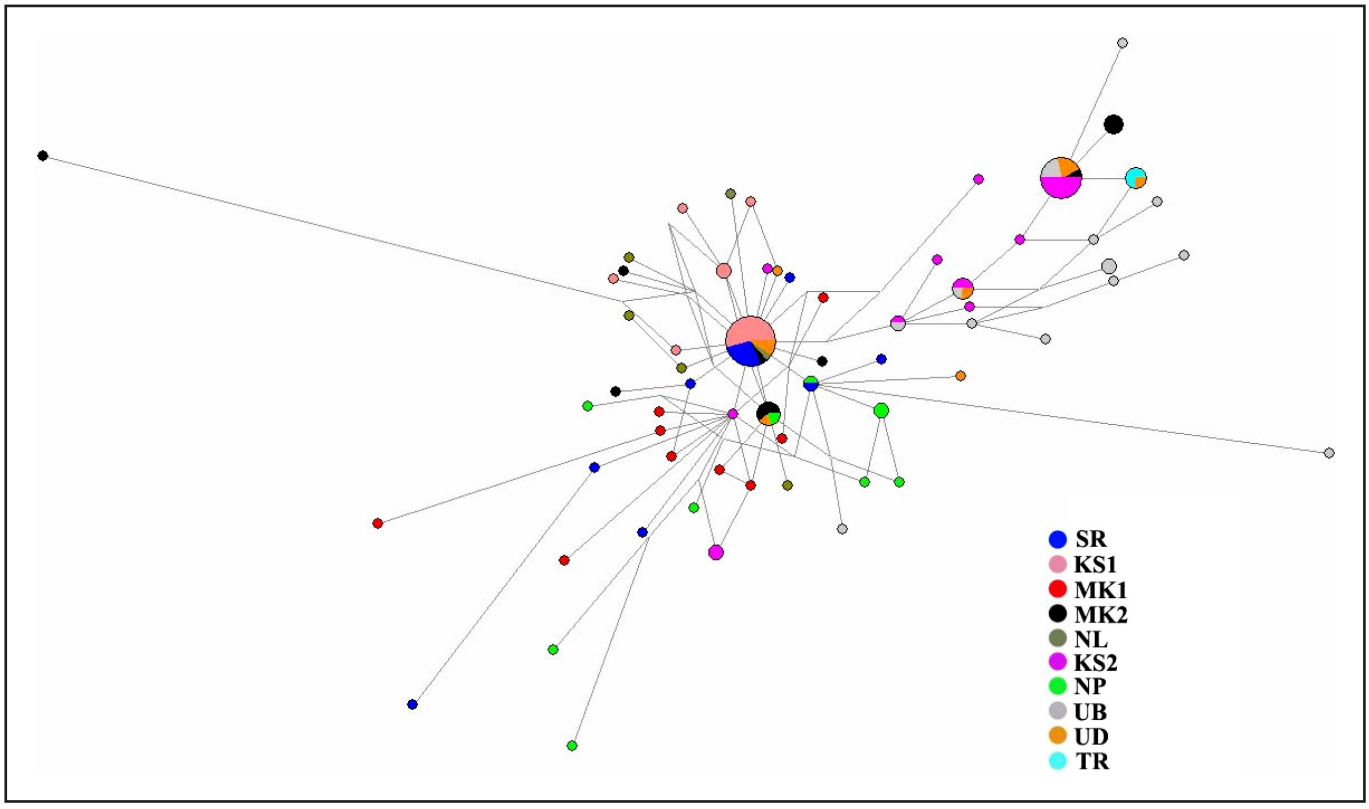

Figure 2. Median joining network of 112 mitochondrial cytochrome *c* oxidase I (COI) sequences of *Culicoides peregrinus* from northeastern Thailand obtained in present study and three COI sequences from southern Thailand (TR) retrieved from Gopurenko *et al*. (2022). Each circle represents a haplotype and sizes are relative to number of individuals sharing such haplotype. Haplotypes are labelled according to the sampling location as in Figure 1.

In contrast to the low level of genetic divergence, population pairwise F_{ST} analysis revealed a surprisingly high level of genetic structuring. Among 45 pairwise comparisons, 32 (71%) were significantly different (Table 2). The many population genetic studies of the *Culicoides* species have usually found low or no genetic structuring, particularly between geographically proximal populations (Calvo *et al*., 2009; Onyango *et al*., 2015a, 2015b; Jacquet *et al*., 2016a, 2016b; Mignotte *et al*., 2021). In Thailand, a population genetic study of the geographically co-distributed and common species, *C. mahasarakhamense*, also found a low level of genetic differentiation between populations (Pramual *et al*., 2022).

The very high level of genetic structuring between populations of *C. peregrinus* is unexpected given that it is among the most abundance and common species, particularly near animal farms. The high level of genetic differentiation among populations of *C. peregrinus* indicated that gene flow is limited. The IBD analysis revealed no significant relationship between level of genetic (i.e. F_{ST} value) and geographic distances ($R^2 = 0.252$, $P = 0.084$). Therefore, geographic distance cannot explain the genetic structuring found in this species. A possible explanation for the high level of genetic differentiation between populations of *C. peregrinus* is that there is limited movement between local population i.e. between cattle pens. The MJ network of the specimens obtained in the present study (Figure 2) indicated more closely related haplotypes within the same population than those from others. Among 64 haplotypes identified in this study, only 6 were shared by >2 populations. This was also supported by the generally low nucleotide diversity in each population. The ability to use diverse types of breeding habitats suggest that immature stages of *C. peregrinus* could be using moist soil around the cattle pens. Furthermore, the generalist feeding habit enables this species to search for host blood sources within or around the cattle pens where different animals including buffalos, cows, chickens and human are available. Taken together, these factors facilitate *C. peregrinus* to complete its life cycle within a cattle pens without any need for searching for host blood sources in other places. This will promote genetic differentiation between local populations.

Although genetic divergence within Thai specimens was relatively low, it was very high (max. p-distance of 5.40%) when sequences from other countries (Australia, China, India, Bangladesh) recorded in GenBank and BOLDs were included. The maximum genetic divergence was found between specimens from Thailand and Australia. This level of genetic divergence is lower than those reported in BOLDs (max. p-distance of 6.25%) for BIN BOLD:AAJ7131 (https://www.boldsystems.org/index.php/Public_BarcodeCluster?clusteruri=BOLD:AAJ7131), a single BIN recorded in BOLDs for *C. peregrinus* thus far (accessed on June 11, 2023). The lower value of genetic divergence in this study compared to BOLD is due to smaller sample size because many sequences from some countries (Indonesia, Timor-Leste, Malaysia, Singapore, Pakistan) used for p-distance calculation in BOLD are not publicly available and thus were not included in our analysis.

Despite the limitations of accessing COI sequences from BOLDs, we have detected cryptic genetic diversity in *C. peregrinus* specimens included in the present study. The MJ network analysis revealed three divergent lineages (A, B, C) (Figure 3) within *C. peregrinus* from Thailand, China, Bangladesh, India and Australia. Specimens from Thailand and China formed lineage A. In fact, five specimens from China shared a haplotype with those from Thailand. Sequences of *C. peregrinus* from Australia formed lineage B and those from India and Bangladesh belonged to lineage C. Genetic differentiation between lineage A (Thailand + China) and B (Australia) ranged between 2.44% and 5.40% and between A and C (India + Bangladesh) ranged between 1.22% – 4.18%. Lower genetic divergence was found between lineage B and C with the p-distance varying between 1.57% and 2.26%.

The type locality of *C. peregrinus* is in India (Wirth & Hubert, 1989), therefore, we assume that the COI sequences reported from that country are more likely to be the true *C. peregrinus*. Genetically divergent lineages of specimens from Thailand + China and Australia from those of India + Bangladesh could either be indicative of different, but morphologically similar species or of genetic structuring within species (Gopurenko *et al*., 2015). Wirth and Hubert (1989) has noted that some females collected from Chonburi province, central Thailand, are different from typical *C. peregrinus* by having more extensive, yellowish pale wing marking. In addition, the pale spot at the base of the mediocubital fork is continuous along veins M3+4 and Cu1 to the wing margin. Our specimens used in this study collected from northeastern Thailand, have the pale wing marking (Figure 4) which agrees with those from central Thailand described in Wirth and Hubert (1989). In addition, based on wing photographs of *C. peregrinus* from China provided in Liu *et al*. (2018) from which the COI sequences (accession nos. KY433456 – 59) were included in present study, these variations of pale wing marking were also found in their specimens. This is in agreement with the COI sequences where Thailand and China belonged to the same lineage. For those specimens of *C. peregrinus* from Australia, according to the wing photograph (Dyce *et al*., 2007), no such variations were found. Based on the COI barcoding sequences and morphological differentiations, we hypothesize that specimens so-called *C. peregrinus* in Thailand and China are different species from those in India and Australia. To test this hypothesis, further study using additional independent molecular marker from nuclear genes is needed to resolve species status of these COI genetically divergent lineages (Gopurenko *et al*., 2015).

Table 2. Population pairwise F_{ST} values between 10 populations of *Culicoides peregrinus* from Thailand

	SR	KS1	MK1	MK2	NL	KS2	NP	UB	UD	TR
SR										
KS1	**0.08472**									
MK1	**0.16539**	**0.36470**								
MK2	**0.13525**	**0.20072**	**0.23096**							
NL	0.07576	0.13991	**0.23445**	0.09923						
KS2	**0.35899**	**0.44416**	**0.39091**	0.09777	**0.35503**					
NP	0.11227	**0.28477**	0.14857	**0.18794**	**0.17016**	**0.37281**				
UB	**0.36576**	**0.47122**	**0.41449**	**0.1779**	**0.36474**	**0.08434**	**0.30978**			
UD	**0.23869**	**0.34084**	**0.33406**	0.00078	**0.22682**	-0.00259	**0.25978**	0.06262		
TR	**0.70778**	**0.85877**	**0.68557**	**0.31396**	0.75177	0.30466	**0.58066**	0.11713	0.31494	

Note: Bold number indicates statistical significance after Bonferroni correction.

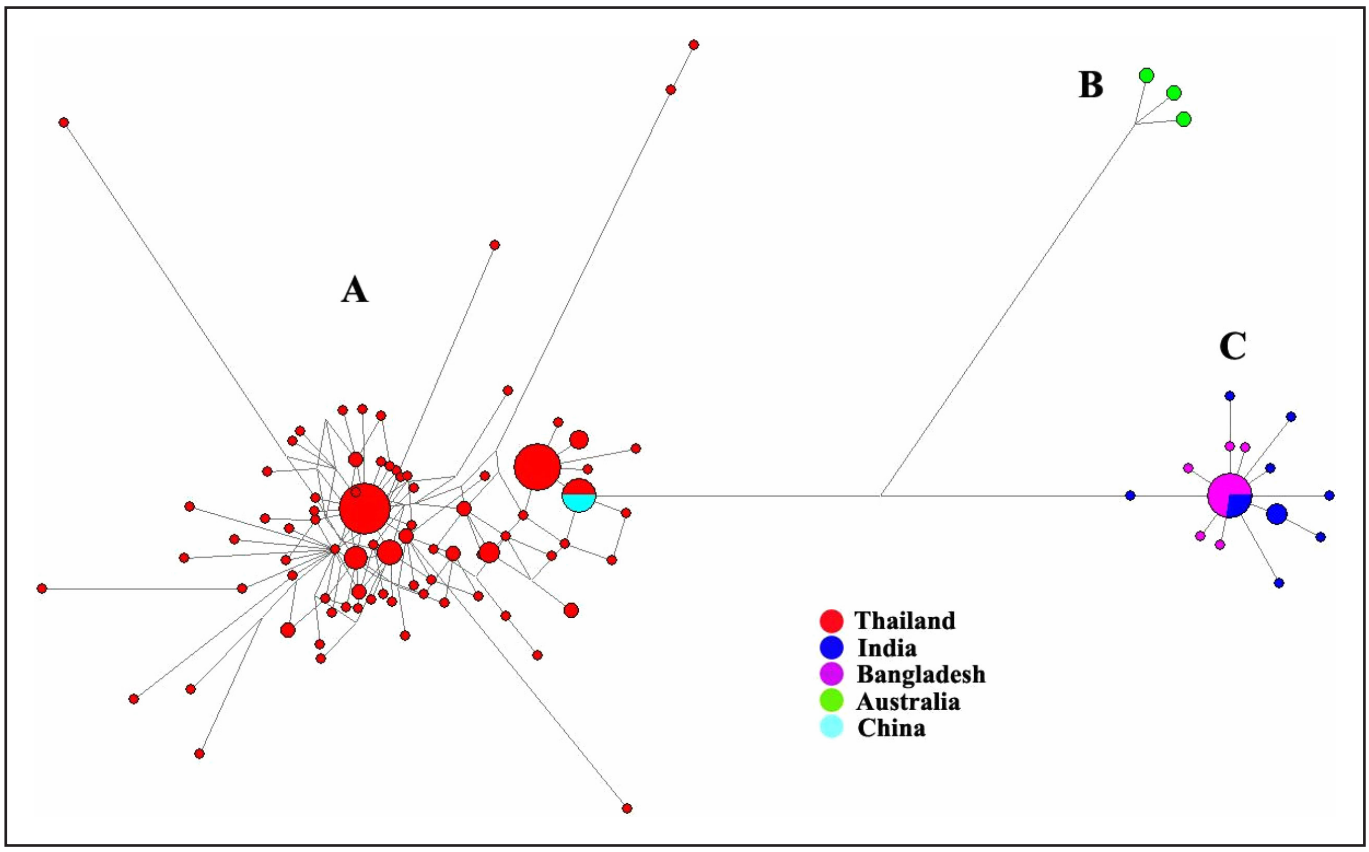

Figure 3. Median joining network of 191 mitochondrial cytochrome *c* oxidase I (COI) sequences of *Culicoides peregrinus* from northeastern Thailand and those reported from other countries reported in public database. Each circle represents a haplotype and sizes are relative to number of individuals sharing such haplotype. Haplotypes are labelled according to the country of origin.

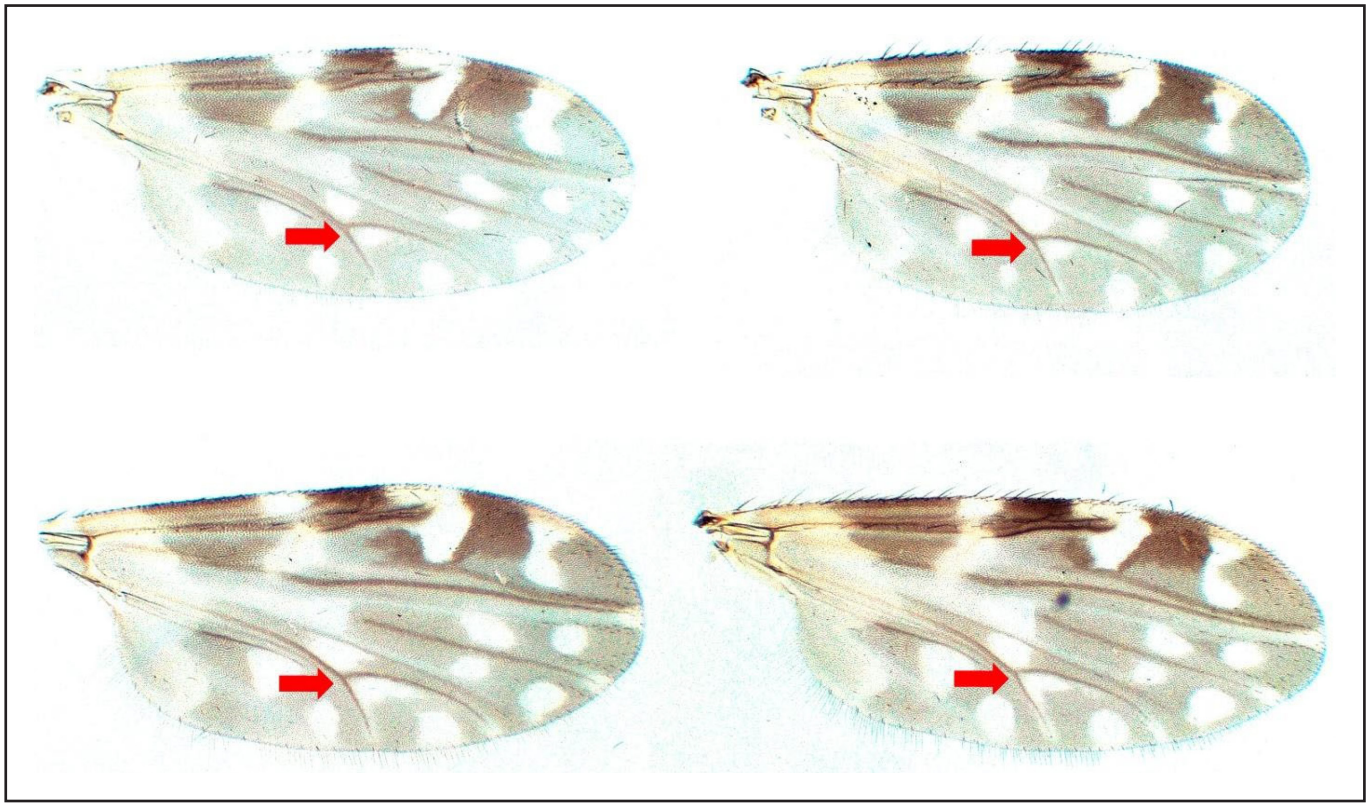

Figure 4. Photographs of female right wings of *Culicoides peregrinus* in northeastern Thailand collected in this study. Arrow indicates variations of the pale spot at the base of mediocubital folk and along the veins M3+4 and Cu1.

In conclusion, we found a high level of genetic structuring within *C. peregrinus* from northeastern Thailand. The ability to utilized diverse breeding habitats as well as a generalist feeding habit could enable *C. peregrinus* to complete its life cycle within cattle pens. This would limit dispersal for host searching or mating and thereby facilitate genetic differentiation even between geographically proximal populations. At the wider geographic scale (i.e. comparisons of specimens from different countries), we found three genetically divergent lineages within *C. peregrinus*. Specimens from Thailand and China belonged to the same lineage and were different from those of India + Bangladesh and Australian lineages. The genetically divergent lineages based on COI sequences were also supported by morphological characteristics. Therefore, these lineages are potentially different, but morphologically similar species. Additional study using the independent genetic markers from nuclear DNA genes will be useful to resolve the species status of *C. peregrinus* recorded in Thailand.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

Ander, M., Troell, K. & Chirico, J. (2013). Barcoding of biting midges in the genus *Culicoides*: a tool for species determination. *Medical and Veterinary Entomology* **27**: 323-331. https://doi.org/10.1111/j.1365-2915.2012.01050.x

Bandelt, H.J., Forster, P. & Röhl, A. (1999). Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**: 37-48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Bohonak, A.J. (2002). IBD (isolation by distance): a program for analyses of isolation by distance. *Journal of Heredity* **93**: 153-154. https://doi.org/10.1093/jhered/93.2.153

Borkent, A. & Dominiak, P. (2020). Catalog of the biting midges of the world (Diptera: Ceratopogonidae). *Zootaxa* **4787**: 1-377. https://doi.org/10.11646/zootaxa.4787.1.1

Calvo, J.H., Calvete, C., Martinez-Royo, A., Estrada, R., Miranda, M.A., Borras, D., Monteys, V.S.I., Pages, N., Delgado, J.A., Collantes, F. *et al*. (2009). Variations in the mitochondrial cytochrome c oxidase subunit I gene indicate northward expanding populations of *Culicoides imicola* in Spain. *Bulletin of Entomological Research* **99**: 583-591. https://doi.org/10.1017/S0007485309006622

Dyce, A.L., Bellis, G.A. & Muller, M.J. (2007). Pictorial Atlas of Australian *Culicoides* Wings (Diptera: Ceratopogonidae). ABRS.

Excoffier, L. & Lischer, H.E. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**: 564-567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 294-299.

Fujisawa, Y., Homat, T., Thepparat, A., Changbunjong, T., Sutummaporn, K., Kornmatitsuk, S. & Kornmatitsuk, B. (2021). DNA barcode identification and molecular detection of bluetongue virus in *Culicoides* biting midges (Diptera: Ceratopogonidae) from western Thailand. *Acta Tropica* **224**: 106147. https://doi.org/10.1016/j.actatropica.2021.106147

Gopurenko, D., Bellis, G., Pengsakul, T., Siriyasatien, P. & Thepparat, A. (2022). DNA barcoding of *Culicoides* Latreille (Diptera: Ceratopogonidae) from Thailand reveals taxonomic inconsistencies and novel diversity among reported sequences. *Journal of Medical Entomology* **59**: 1960-1970. https://doi.org/10.1093/jme/tjac142

Gopurenko, D., Bellis, G.A., Yanase, T., Wardhana, A.H., Thepparat, A., Wang, J., Li, H., Cai, D. & Mitchell, A. (2015). Integrative taxonomy to investigate species boundaries within *Culicoides* (Diptera: Ceratopogonidae): a case study using subgenus *Avaritia* from Australasia and Eastern Asia. *Veterinaria Italiana* **51**: 345-378. https://doi.org/10.12834/VetIt.515.2463.2

Harrup, L.E., Bellis, G.A., Balenghien, T. & Garros, C. (2015). *Culicoides* Latreille (Diptera: Ceratopogonidae) taxonomy: current challenges and future directions. *Infection, Genetics and Evolution* **30**: 249-266. https://doi.org/10.1016/j.meegid.2014.12.018

Harrup, L.E., Laban, S., Purse, B.V., Reddy, Y.K., Reddy, Y.N., Byregowda, S.M., Kumar, N., Purushotham, K.M., Kowalli, S., Prasad, M., Prasad, G. *et al*. (2016). DNA barcoding and surveillance sampling strategies for *Culicoides* biting midges (Diptera: Ceratopogonidae) in southern India. *Parasites & Vectors* **9**: 461. https://doi.org/10.1186/s13071-016-1722-z

Harsha, R. & Mazumdar, A. (2015). Laboratory rearing of immature *Culicoides peregrinus* Kieffer, a potential vector of bluetongue virus. *Medical and Veterinary Entomology* **29**: 434-438. https://doi.org/10.1111/mve.12136

Harsha, R., Mazumdar, S.M. & Mazumdar, A. (2020). Abundance, diversity and temporal activity of adult *Culicoides* spp. associated with cattle in West Bengal, India. *Medical and Veterinary Entomology* **34**: 327-343. https://doi.org/10.1111/mve.12446

Jacquet, S., Huber, K., Guis, H., Setier-Rio, M.L., Goffredo, M., Allɒne, X., Rakotoarivony, I., Chevillon, C., Bouyer, J., Baldet, T. *et al*. (2016a). Spatio-temporal genetic variation of the biting midge vector species *Culicoides imicola* (Ceratopogonidae) Kieffer in France. *Parasites & Vectors* **9**: 141. https://doi.org/10.1186/s13071-016-1426-4

Jacquet, S., Huber, K., Pagɒs, N., Talavera, S., Burgin, L.E., Carpenter, S., Sanders, C., Dicko, A.H., Djerbal, M., Goffredo, M. *et al*. (2016b). Range expansion of the Bluetongue vector, *Culicoides imicola*, in continental France likely due to rare wind-transport events. *Scientific Reports* **6**: 27247. https://doi.org/10.1038/srep27247

Jomkumsing, P., Surapinit, A., Saengpara, T. & Pramual, P. (2021). Genetic variation, DNA barcoding and blood meal identification of *Culicoides* Latreille biting midges (Diptera: Ceratopogonidae) in Thailand. *Acta Tropica* **217**: 105866. https://doi.org/10.1016/j.actatropica.2021.105866

Kar, S., Mondal, B., Ghosh, J., Mazumdar, S.M. & Mazumdar, A. (2022a). Host preference of bluetongue virus vectors, *Culicoides* species associated with livestock in West Bengal, India: Potential relevance on bluetongue epidemiology. *Acta Tropica* **235**: 106648. https://doi.org/10.1016/j.actatropica.2022.106648

Kar, S., Mondal, B., Pal, A., Harsha, R. & Mazumdar, A. (2022b). Blood meal analysis of *Culicoides* species associated with livestock in West Bengal, India. *Medical and Veterinary Entomology* **36**: 503-510. https://doi.org/10.1111/mve.12588

Kamyingkird, K., Choocherd, S., Chimnoi, W., Klinkaew, N., Kengradomkij, C., Phoosangwalthong, P., Thammasonthijarern, N., Pattanatanang, K., Inpankaew, T., Phasuk, J. *et al*. (2023). Molecular identification of *Culicoides* species and host preference blood meal in the African horse sickness outbreak-affected area in Hua Hin district, Prachuap Khiri Khan province, Thailand. *Insects* **14**: 369.

Liu, Y., Tao, H., Yu, Y., Yue, L., Xia, W., Zheng, W., Ma, H., Liu, X. & Chen, H. (2018). Molecular differentiation and species composition of genus *Culicoides* biting midges (Diptera: Ceratopogonidae) in different habitats in southern China. *Veterinary Parasitology* **254**: 49-57. https://doi.org/10.1016/j.vetpar.2018.02.035

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**: 209-220.

McCoy, K.D. (2008). The population genetic structure of vectors and our understanding of disease epidemiology. *Parasite* **15**: 444-448. https://doi.org/10.1051/parasite/2008153444

Meier, R., Shiyang, K., Vaidya, G. & Ng, P.K. (2006). DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Systematic Biology* **55**: 715-728. https://doi.org/10.1080/10635150600969864

Mignotte, A., Garros, C., Dellicour, S., Jacquot, M., Gilbert, M., Gardès, L., Balenghien, T., Duhayon, M., Rakotoarivony, I., de Wavrechin, M. *et al*. (2021). High dispersal capacity of *Culicoides obsoletus* (Diptera: Ceratopogonidae), vector of bluetongue and Schmallenberg viruses, revealed by landscape genetic analyses. *Parasites & Vectors* **14**: 93. https://doi.org/10.1186/s13071-020-04522-3

Mullen, G.R. & Murphree, C.S. (2019). Biting midges (Ceratopogonidae). In: Medical and Veterinary Entomology, Mullen, G.R. & Durden, L.A. (editors), 3rd edition. San Diego, CA: Elsevier, pp. 213-236. https://doi.org/10.1016/B978-0-12-814043-7.00013-3

Onyango, M.G., Beebe, N.W., Gopurenko, D., Bellis, G., Nicholas, A., Ogugo, M., Djikeng, A., Kemp, S., Walker, P.J. & Duchemin, J.B. (2015a). Assessment of population genetic structure in the arbovirus vector midge, *Culicoides brevitarsis* (Diptera: Ceratopogonidae), using multi-locus DNA microsatellites. *Veterinary Research* **46**: 108. https://doi.org/10.1186/s13567-015-0250-8

Onyango, M.G., Michuki, G.N., Ogugo, M., Venter, G.J., Miranda, M.A., Elissa, N., Djikeng, A., Kemp, S., Walker, P.J. & Duchemin, J.B. (2015b). Delineation of the population genetic structure of *Culicoides imicola* in East and South Africa. *Parasites & Vectors* **8**: 660. https://doi.org/10.1186/s13071-015-1277-4

Powell, J.R. (2018). Genetic variation in insect vectors: death of typology? *Insects* **9**: 139. https://doi.org/10.3390/insects9040139

Pramual, P., Jomkumsing, P., Jumpato, W. & Bunauea, S. (2021a). Molecular detection of avian haemosporidian parasites in biting midges (Diptera: Ceratopogonidae) from Thailand. *Acta Tropica* **224**: 106118. https://doi.org/10.1016/j.actatropica.2021.106118

Pramual, P., Jomkumsing, P., Piraonapicha, K. & Jumpato, W. (2021b). Integrative taxonomy uncovers a new *Culicoides* (Diptera: Ceratopogonidae) biting midge species from Thailand. *Acta Tropica* **220**: 105941. https://doi.org/10.1016/j.actatropica.2021.105941

Pramual, P., Jomkumsing, P., Wongpakam, K., Vaisusuk, K., Chatan, W. & Gomontean, B. (2022). Population genetic structure and population history of the biting midge *Culicoides mahasarakhamense* (Diptera: Ceratopogonidae). *Insects* **13**: 724. https://www.mdpi.com/2075-4450/13/8/724#

Ranjan, K., Prasad, M., Brar, B. & Prasad, G. (2017). First report of isolation of bluetongue virus 23 from *Culicoides peregrinus* vector from India. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* **38**: 16-21. http://10.0.23.70/0974-0147.2017.00003.4

Sen, P. & Gupta, S.D. (1959). Studies on Indian *Culicoides* (Ceratopogonidae: Diptera). *Annals of the Entomological Society of America* **52**: 617-630. https://doi.org/10.1093/aesa/52.5.617

Songumpai, N., Promrangsee, C., Noopetch, P., Siriyasatien, P. & Preativatanyou, K. (2022). First evidence of co-circulation of emerging *Leishmania martiniquensis*, *Leishmania orientalis*, and *Crithidia* sp. in *Culicoides* biting midges (Diptera: Ceratopogonidae), the putative vectors for autochthonous transmission in Southern Thailand. *Tropical Medicine and Infectious Disease* **7**: 379.

Sunantaraporn, S., Hortiwakul, T., Kraivichian, K., Siriyasatien, P. & Brownell, N. (2022). Molecular identification of host blood meals and detection of blood parasites in *Culicoides* Latreille (Diptera: Ceratopogonidae) collected from Phatthalung Province, Southern Thailand. *Insects* **13**: 912.

Sunantaraporn, S., Thepparat, A., Phumee, A., Sor-Suwan, S., Boonserm, R., Bellis, G. & Siriyasatien, P. (2021). *Culicoides* Latreille (Diptera: Ceratopogonidae) as potential vectors for *Leishmania martiniquensis* and *Trypanosoma* sp. in northern Thailand. *PLoS Neglected Tropical Diseases* **15**: e0010014. https://doi.org/10.1371/journal.pntd.0010014

Tabachnick, W.J. & Black IV, W. (1995). Making a case for molecular population genetic studies of arthropod vectors. *Parasitology Today* **11**: 27-30. https://doi.org/10.1016/0169-4758(95)80105-7

Tangkawanit, U., Wongpakam, K. & Pramual, P. (2018). A new black fly (Diptera: Simuliidae) species of the subgenus *Asiosimulium* Takaoka Choochote from Thailand. *Zootaxa* **4388**: 111-122. https://doi.org/10.11646/zootaxa.4388.1.8

Wirth, W.W. & Hubert, A.A. (1989). The Culicoides of Southeast Asia (Diptera: Ceratopogonidae). *Memoirs of the American Entomological Institute* **44**: 1-508.

Yanase, T., Matsumoto, Y., Matsumori, Y., Aizawa, M., Hirata, M., Kato, T., Shirafuji, H., Yamakawa, M., Tsuda, T. & Noda, H. (2013). Molecular identification of field-collected *Culicoides* larvae in the southern part of Japan. *Journal of Medical Entomology* **50**: 1105-1110. https://doi.org/10.1603/ME11235