RESEARCH ARTICLE

Population genetics of the biting midge *Culicoides oxystoma* Kieffer (Diptera: Ceratopogonidae) from Thailand and its genetic relationships with global populations

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ABSTRACT

Culicoides oxystoma Kieffer is a vector of viruses, filarial nematodes and protozoa of the genus *Leishmania* transmitted to humans and other animals. Understanding genetic diversity, genetic structure and genetic relationships among geographically widespread populations will provide important information related to disease epidemiology. In this study, genetic diversity, genetic structure and genetic relationships between Thai *C. oxystoma* and those reported from other countries were inferred based on mitochondrial cytochrome oxidase I (COI) and nuclear internal transcribed spacer 1 (ITS-1) sequences. A high level of genetic diversity was found in *C. oxystoma* from Thailand. The maximum K2P intraspecific genetic divergence for COI gene and ITS-1 sequences were 4.29% and 6.55%, respectively. Despite high genetic diversity, no significant genetic differentiation was found within the 13 Thai populations. This could be a result of unspecialized habitat requirement of the larval habitat, abundance and continuous distribution of host blood sources, potential for long distance movement with host via trading. Mitochondrial genealogy analysis of the global population of *C. oxystoma* revealed three (A, B and C) genetically divergent lineages. Specimens from Thailand were included in the main lineage (A) with those from all other countries except those from Senegal that formed lineage B and those of Lineage C that was exclusively found in Bangladesh. The nuclear (ITS-1) genetic markers genealogy indicated that Thai *C. oxystoma* belong to the same genetic lineage with those from East, South and Southeast Asia which presumably the true *C. oxystoma*.

Keywords: Biting midge; genetic diversity; genetic structure; insect vector.

INRODUCTION

Several species of the biting midge genus *Culicoides* Latreille (Diptera: Ceratopogonidae) are important pests and vectors of pathogens including viruses, protozoans, and nematodes that are transmitted to humans and other animals. Significant diseases in which biting midge species are involved as vectors include Oropouche fever in humans, bluetongue disease in ruminants, African horse sickness in equines, and leucocytozoonosis in chickens (Valkiunas, 2005; Mullen & Murphree, 2019). In addition to having significant impact on human and animal welfare, disease caused by the pathogens transmitted by biting midge species can cause severe economic losses. For examples, the bluetongue virus (BTV) transmitted by at least seven biting midge species has now been found across the globe (Mullen & Murphree, 2019). It has been estimated that the economic impact as a consequence of a BTV outbreak in France was more than 1.4 billion USD (Walker, 2009). The African Horse Sickness Virus (AHSV) transmitted by *C. imicola* Kieffer causing African

Horse Sickness disease has impacted the equine industry by approximately 95 million USD a year (Redmond *et al.*, 2022). Recently (February 2020), more than 500 horse deaths in Thailand cause of an outbreak of this disease (Castillo Olivares, 2021).

The control program for vector-borne disease requires full understanding of biodiversity of the vector species which can be obtained using a population genetic approach. Knowledge of the genetic diversity, genetic structure and population history of the vector species is crucial because this information can be used for effective vector control (Tabachnick & Black, 1995; Pᵣ rez De Rosas *et al.*, 2007; McCoy, 2008). For example, the level of genetic differentiation, rate and direction of gene flows can be used to determine the spread of pathogens or sources of the parasite and vector incursions (Onyango *et al.*, 2015; Jacquet *et al.*, 2016). Understanding the level of genetic differentiation between populations is helpful for determining the species status of the vector. This information is potentially related to the vector competency (McCoy, 2008).

Culicoides oxystoma Kieffer belongs to the subgenus *Remmia* Glukhova (Borkent & Dominak, 2020) and is one of the most significant pest and vector species of the genus *Culicoides*. There are many pathogens that are transmitted or potentially transmitted by *C. oxystoma* including Akabane virus (Yanase, 2005), bluetongue virus (Dadawala *et al.*, 2012; Fujisawa *et al.*, 2021), Kasba virus (Mullen & Murphree, 2019), filarial nematode (*Onchocerca gibsoni*) (Wirth & Hubert, 1989) and protozoa of the genus *Leishmania* (Songumpai *et al.*, 2022). *Culicoides oxystoma* was originally described from Kolkata, India (Wirth & Hubert, 1989) and is now known to be widely distributed from Africa to Southeast Asia, China and Japan (Wirth & Hubert, 1989; Bakhoum *et al.*, 2013; Liu *et al.*, 2018; Slama *et al.*, 2021). This wide distribution possibly relates to the unspecialized habitat requirement of the larva. Morphologically, *C. oxystoma* show great variability in wing pattern, mesonotal pattern and in the form of the male terminalia (Wirth & Hubert, 1989). *C. oxystoma* is also genetically highly variable, particularly when comparing amongst populations from different geographic regions. For examples, genetic divergence between Senegalese *C. oxystoma* and populations from Australia, Israel and Japan showed >4% differentiation (Bakhoum *et al.*, 2018). Similar figures (3.7–5.8%) have been reported for the comparison of Indian and Senegalese *C. oxystoma*. This geographically associated genetic differentiation was hypothesized to reflect cryptic diversity within this species (Bakhoum *et al.*, 2018) or misidentification (Rot *et al.*, 2020).

In Thailand, *C. oxystoma* have been recorded throughout the country (Wirth & Hubert, 1989; Fujisawa *et al.*, 2021; Pramual *et al.*, 2021; Sunantaraporn *et al.*, 2021; Songumpai *et al.*, 2022). It is commonly and dominantly found in or close to animal shelters (Sunantaraporn *et al.*, 2021). Molecular identification of the host blood sources found that this species bites cattle and buffalo (Jomkumsing *et al.*, 2021). Recently, *C. oxystoma* collected in western Thailand has been suspected as a potential vector of bluetongue virus (Fujisawa *et al.*, 2021) while specimens from the south are possible vectors of *Leishmania martiniquensis* and *L. orientalis*, the causative agents of Leishmaniasis and with the highest infection rate (10.6%) compared to other *Culicoides* species (*C. huffi*, 4%; *C. peregrinus*, 9.1%; *C. mahasarakhamense*, 1.5%) (Songumpai *et al.*, 2022). Therefore, *C. oxystoma* in Thailand is potentially a significant vector transmitting disease causing agents to humans and other animals. Understanding genetic structure and diversity of this species will be very useful for fully understanding the epidemiology of the diseases (McCoy, 2008) potentially transmitted by *C. oxystoma*. The pathogens (BTV, *Leishmania* spp.) detected in *C. oxystoma* in Thailand are geographically restricted i.e. BTV is present in Kanchanaburi province in the western region (Fujisawa *et al.*, 2021), while *Lieshmania* has been found in Songkhla province in the southern region (Songumpai *et al.*, 2022); therefore, knowledge of genetic differentiation between populations of possible vector species will be useful for monitoring the spread of the pathogens.

In this study, a population genetic approach was used to examine genetic diversity and genetic structure of *C. oxystoma* in Thailand based on the mitochondrial cytochrome oxidase I (COI) and internal transcribed spacer 1 (ITS-1) sequences. A previous DNA barcoding study found a considerable level of genetic divergence (max. 3.45%) (Jomkumsing *et al.*, 2021) suggesting possible cryptic diversity which could be examined using a population genetic approach. In addition, comparisons with the DNA barcoding sequences reported from other countries found very high level of genetic divergence (max. 8.51%) (Jomkumsing *et al.*, 2021). Therefore, it will also be important to examine genetic relationships amongst Thai *C. oxystoma* with those from other geographic regions. This approach can be used to assess the species status of this significant pest and potential vector (Morag *et al.*, 2012).

MATERIALS AND METHODS

Specimen collections and identification
Adult specimens of *C. oxystoma* were collected from 12 sampling sites in Thailand between November 2020 and February 2021 (Table 1 and Figure 1). Data on three additional populations were obtained from previous publications (Fujisawa *et al.*, 2021; Jomkumsing *et al.*, 2021; Sunantaraporn *et al.*, 2021). Specimens were sampled at or near cattle pens where the biting midges were abundant using a sweep-net for sweeping around animals and also randomly in the air. Specimens were collected between 17:00 and 19:00 pm as biting midges are actively searching for a host blood meal during this time period. The adult flies were preserved in 80% ethanol and stored at -20°C until used. Specimens were identified using descriptions of Wirth and Hubert (Wirth & Hubert, 1989). Examples of variations in the wing patterns of female *C. oxystoma* specimens used in this study are shown in Figure 2. There is no geographic association among these different wing patterns. In total, 9,663 biting midge specimens were collected from 13 sampling sites, with 3,982 (41%) identified as *C. oxystoma*. The percentage of *C. oxystoma* in each sampling location varied between 2% and 89%.

DNA extraction, polymerase chain reaction (PCR), and sequencing
A total of 132 adult specimens of *C. oxystoma* were used for molecular study. DNA was extracted from a single individual specimen for each extraction using the GF-1 Nucleic Acid Extraction Kit (Vivantis Technologies Sdn. Bhd, Malaysia). The mitochondrial cytochrome c oxidase I (COI) gene barcoding region was amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994). The PCR reaction conditions were as described in Tangkawanit *et al.* (2018). In addition to the COI gene, we also used a subset of specimens (60 individuals) for nuclear marker study based on the internal transcribed spacer 1 (ITS-1) sequences. An approximately 500 bp fragment was amplified using primers PanCulF (5'-GTAGGTGAACCTGCGGAAGG-3') and PanCulR (5'-TGCGGTCTTCATCGACCCAT-3') (Cêtre-Sossah *et al.*, 2004). The PCR reaction conditions followed Morag *et al.* (2012). PCR products were checked with 1% agarose gel electrophoresis. A PureDireX PCR CleanUp & Gel Extraction Kit (BioHelix, Taiwan) was used for purification of the PCR products before sequencing at the ATCG Company Limited (Thailand Science Park (TSP), Pathumthani, Thailand) using the same primers as for PCR.

Data analysis
For the COI gene, a total of 161 sequences from Thai specimens, each with a sequence length of 534 bp, were used for genetic diversity, genetic structure and population demographic history analyses. Among these sequences, 132 were obtained in the present study (GenBank accession nos. OQ535929 - OQ536060) and 29 were retrieved from previous publications (Fujisawa *et al.*, 2021; Jomkumsing *et al.*, 2021; Sunantaraporn *et al.*, 2021). For the ITS-1, 43 sequences (GenBank accession nos. OR587863-OR587905) were obtained from Thai specimens in present study. Additionally, 13 sequences from other countries (Israel, accession nos. JN408469–73, JN408476, JN408478–79; India, accession nos. ON806535–6; Japan, accession no. AB462279; Nigeria, accession nos. OM459833–5) were retrieved from GenBank. The ITS-1 sequences from a closely related species, *C. schultzei* reported from India (accession no. KY458555) and Israel (accession nos. JN408474–5, JN408477) were also included in data analyses.

Genetic diversity indices based on haplotype diversity (h) and nucleotide diversity (π) were calculated using the K2P model in Arlequin ver. 3.5 (Excoffier & Lischer, 2010). Intraspecific

Table 1. Sampling location and number of specimens of *Culicoides oxystoma* in Thailand used in this study

Location (Code)	Latitude / Longitude	Elevation (m)	N (h_n)	h_d ± S.D.	π ± S.D.	Collection Date
Mahasarakham University, Maha Sarakham (MK1)	16.2488° N / 103.2505° E	150	25^1 (25)	1.0000 ± 0.0113	0.0212 ± 0.0111	25 Feb 2019 (previous studied)
Ban Don Wiangchan, Maha Sarakham (MK2)	16.2566° N / 103.2663° E	160	5 (5)	1.0000 ± 0.1265	0.0166 ± 0.0108	25 Mar 2021
Huai Mek, Kalasin (KL)	16.6055° N / 103.2238° E	190	16 (16)	0.9917 ± 0.0254	0.0180 ± 0.0098	23 Jan 2021
Non Sang, Nongbualamphu (NL)	16.8233° N / 102.5688° E	180	6 (6)	1.0000 ± 0.0962	0.0234 ± 0.0143	27 Feb 2021
Waritchaphum, Sakon Nakhon (SN)	17.2422° N / 103.5744° E	220	5 (5)	1.0000 ± 0.1265	0.0222 ± 0.0142	27 Mar 2021
Phon Sawan, Nakhon Phanom (NP)	17.4616° N / 104.4658° E	150	10 (10)	1.0000 ± 0.0447	0.0167 ± 0.0095	20 Nov 2020
Lerng Noktha, Yasothon (YT)	16.2611° N / 104.5227° E	160	10 (9)	0.9778 ± 0.0540	0.0177 ± 0.0100	14 Apr 2021
Prangku, Sisaket (SK)	14.8305° N / 104.0605° E	140	14 (14)	1.0000 ± 0.0270	0.0228 ± 0.0123	4 Jun 2022
Kud Khaopun, Ubon Ratchathani (UB1)	15.7330° N / 104.9680° E	130	13 (12)	0.9872 ± 0.0354	0.0200 ± 0.0110	13 Jan 2021
Khongchiam, Ubon Ratchathani (UB2)	15.3163° N / 105.5127° E	110	13 (12)	0.9872 ± 0.0354	0.0160 ± 0.0089	11 Nov 2022
Tha Sala, Nakhon Si Thammarat (NT)	8.6402° N / 99.9036° E	80	6 (6)	1.0000 ± 0.0962	0.0183 ± 0.0113	20 Sep 2022
Phu Ruea, Loei (LO)	17.4308° N / 101.3500° E	650	16 (15)	0.9917 ± 0.0254	0.0149 ± 0.0082	20 Jan 2021
Mueang Nong Khai, Nong Khai (NK)	17.7841° N / 102.6630° E	180	18 (17)	0.9935 ± 0.0210	0.0174 ± 0.0094	2 Jul 2022
Lamphun	N/A	N/A	3^2 (3)	1.0000 ± 0.2722	0.0183 ± 0.0145	N/A (previous studied)
Kanchanaburi	N/A	N/A	1^3 (1)	N/A	N/A	N/A (previous studied)
Total			**161 (145)**	**0.9980 ± 0.0012**	**0.0194 ± 0.0099**	

Data from [1]Jomkumsing *et al.* (2021); [2]Sunantaraporn *et al.* (2021); [3]Fujisawa *et al.* (2021).
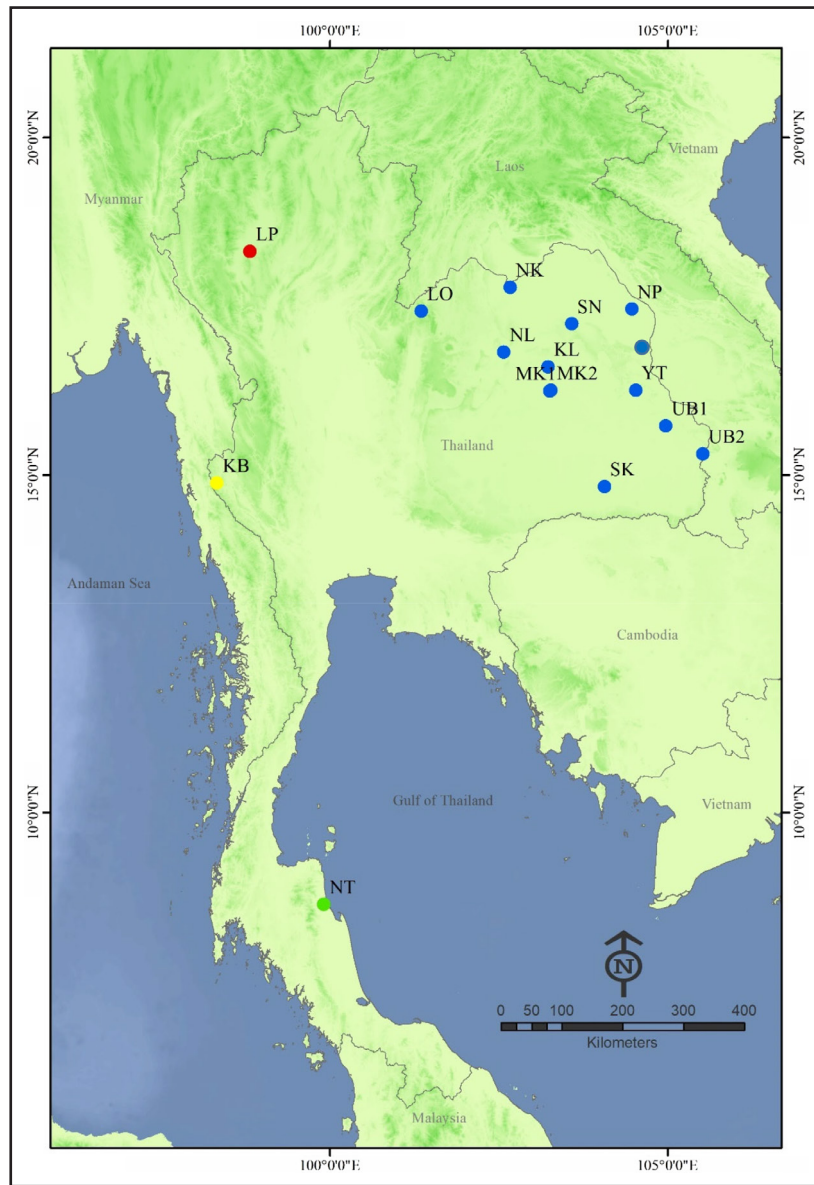
127

Figure 1. Map of Thailand indicate the 15 sampling locations of *Culicoides oxystoma* in this study. Details of sampling locations are included in Table 1. Locality symbols are labeled according to geographic region: red, north; blue, northeast; yellow, west; green, south.
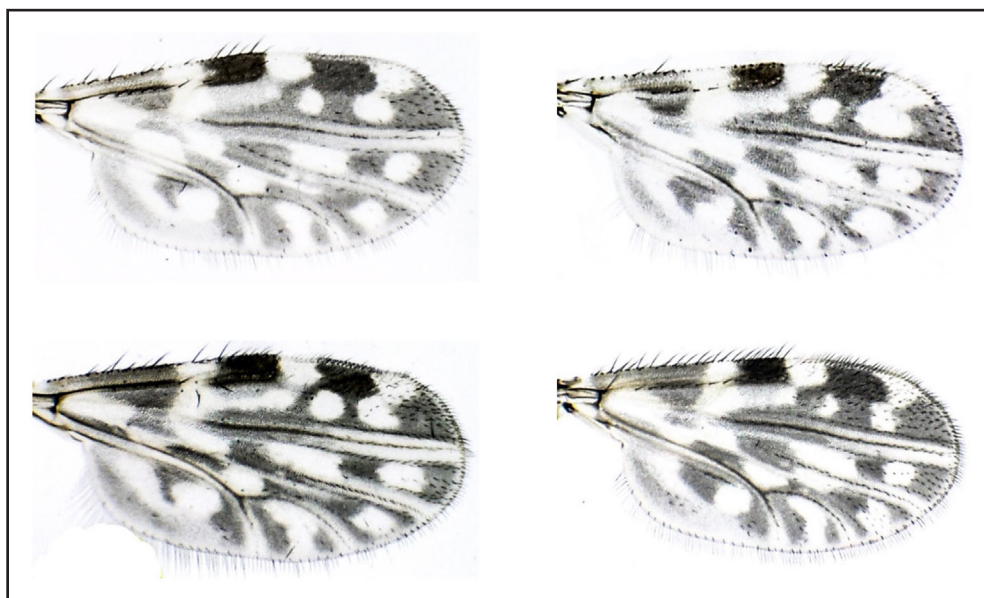

Figure 2. Photographs of right wing of specimens of *Culicoides oxystoma* in Thailand used in this study.

genetic divergence based on the K2P model calculated in TaxonDNA (Meier *et al.*, 2006) was also used to indicate the level of genetic diversity. Genetic differentiation between populations based on pairwise F_{ST} was calculated in Arlequin using 1023 permutations for each statistical test. The sequential Bonferroni correction was used to adjust the significance level for multiple tests. To avoid bias as a result of a small sampling size, populations with sample sizes of less than five were not included in the F_{ST} analysis. A median joining (MJ) network was used to infer genetic relationships between the COI haplotypes. MJ network analysis was performed in the software Network ver. 10.2.0.0 (https://www.fluxus-engineering.com).

To infer the genetic relationships between *C. oxystoma* in Thailand and those from other geographic regions, MJ network analysis was used. Of a total of 1,037 COI sequences analyzed, 132 were obtained in the present study and 905 were retrieved from public databases (NCBI GenBank and BOLDs (https://www.boldsystems.org). MJ network analysis was performed in Network ver. 10.2.0.0 (https://www.fluxus-engineering.com). Genetic divergence within and between genetic lineages identified by the MJ network were calculated in TaxonDNA (Meier *et al.*, 2006) using the K2P model.

RESULTS

Genetic diversity of *Culicoides oxystoma* in Thailand
The K2P intraspecific genetic divergence of *C. oxystoma* in Thailand based on the COI gene sequences varied between 0% and 4.29% with an average of 1.94%. Nucleotide diversity within each population varied between 0.0149 in the LO population and 0.0234 in the NL population (see Table 1 for locality abbreviations) and the overall nucleotide diversity was 0.0194. There was a high level of haplotype diversity within Thai *C. oxystoma* with a total of 145 haplotypes identified among 161 specimens. The overall level of haplotype diversity was 0.9980 varying from 0.9778 in YT and 1 in eight populations (MK1, MK2, NL, SN, NP, SK, NT and LP) (Table 1). A total of 41 haplotypes were identified among 43 ITS-1 sequences. Intraspecific genetic divergence based on ITS-1 sequences for Thai specimens varied between 0% and 6.55% with an average of 2.92%.

Genetic structure and DNA genealogy of *Cuclicoides oxystoma* in Thailand
Population pairwise F_{ST} values between 13 populations of *C. oxystoma* from Thailand based on the COI gene sequences were low (<0.178) and all comparisons were not statistically significantly different (Figure 3). This overall low level of genetic structuring is agreement with the median joining network as there was no indication of any phylogeographic break or the genetic association of the lineage with the geography (Figure 4a). The MJ network based on ITS-1 sequences also supported a low genetic structure indicated by the COI MJ network as there is no major genetic break (Figure 4b).
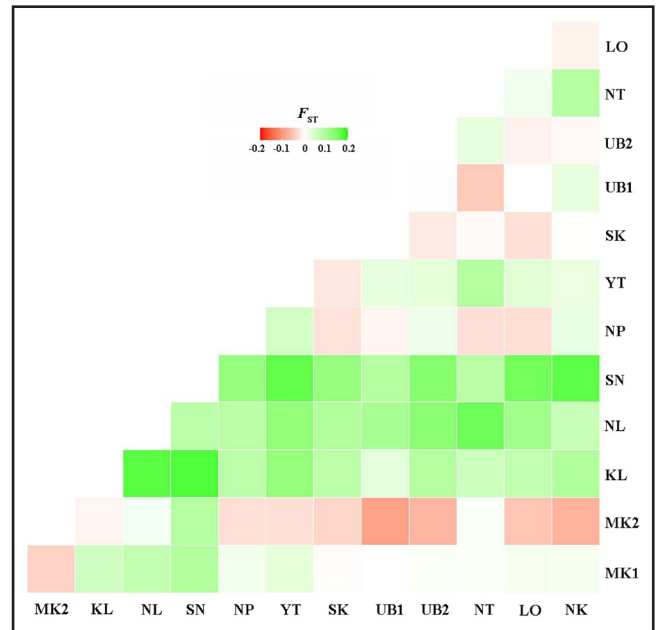

Figure 3. Heat map for the pairwise F_{ST} values between 13 populations of *Culicoides oxystoma* in Thailand calculated based on COI sequences using the Kimura 2-parameter model. Details of populations are provided in Table 1.
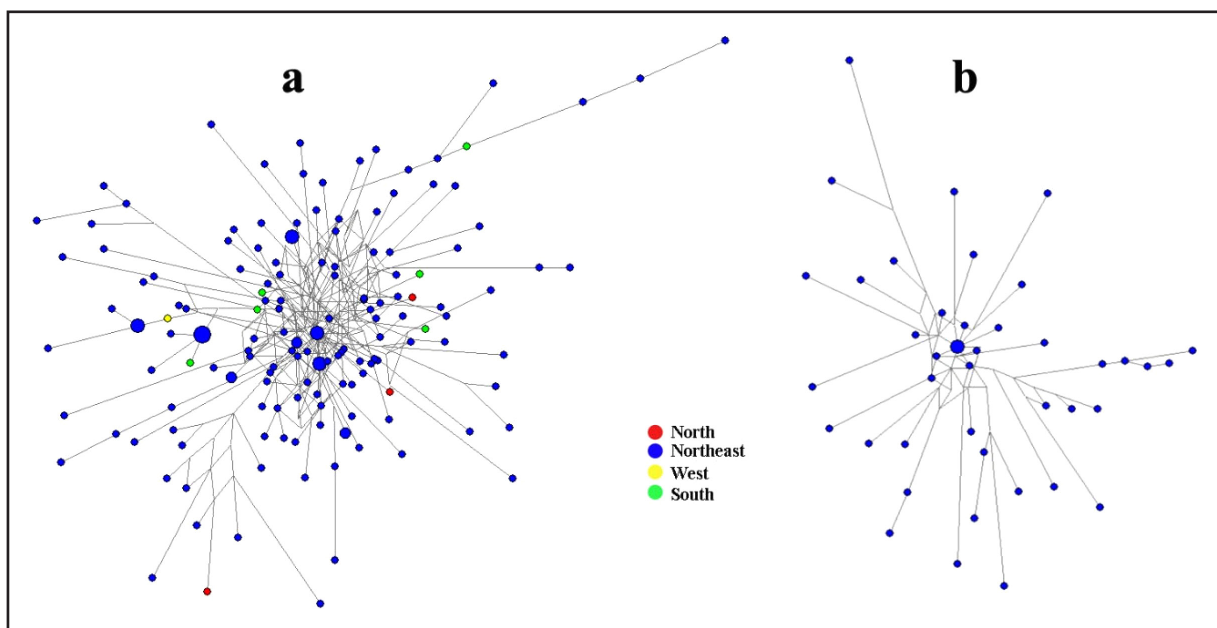

Figure 4. Median joining network of (a) 161 mitochondrial cytochrome c oxidase I (COI) sequences and (b) 43 ITS-1 sequences of *Culicoides oxystoma* in Thailand. Each circle represents a haplotype and sizes are relative to the number of individuals sharing such haplotypes. Haplotypes are labelled according to the geographic regions.

Global genetic diversity and genealogy of *Culicoides oxystoma*

The global genetic diversity of *C. oxystoma* calculated from 1,037 COI sequences (132 from present study and 905 from public databases (NCBI GenBank, BOLDs) varied between 0% and 22.43% with an average of 3.14%. Maximum intraspecific genetic divergence was found between specimens collected in Senegal reported in BOLDs. However, if the extremely divergent (>10%) haplotypes (5 haplotypes, two from Senegal, two from India and one from Kenya) were removed, the maximum intraspecific genetic divergence within species was 10.03% with an average of 3.02%. Because these extremely divergent sequences were potentially due to species misidentification, we omitted them from further analysis.

Intraspecific genetic divergence based on the ITS-1 sequences from other countries (*n* = 56) (Israel, accession nos. JN408469–73, JN408476, JN408478–79), India (accession nos. ON806535–6), Japan (accession no. AB462279) and Nigeria (accession nos. OM459833–5)) revealed very high intraspecific genetic divergence with a maximum value of 16.41%. The high level of genetic divergence within *C. oxystoma* based on ITS-1 sequences was due to the genetically highly divergent sequences from Nigeria (>9.49%) compared to those from other countries.

The median joining network inferred from 1,032 COI sequences of *C. oxystoma* revealed three divergent genetic lineages (A, B and C) (Figure 5a). Lineage A was comprised of *C. oxystoma* from several countries; Thailand, India, Bangladesh, China, Japan, Pakistan, Lebanon, Egypt and Israel. Lineage B was comprised mostly of the

C. oxystoma from Senegal, except one sequence from China and two sequences from Mali that shared haplotypes with those of specimens from Senegal. Lineage C was exclusively representative of the specimens from Bangladesh. Lineage A that comprised specimens from several countries showed a relatively high level of K2P intraspecific genetic divergence with a maximum value of 6.67% and an average of 2.11%. Lineage B that was comprised almost entirely of specimens from Senegal showing the highest intraspecific genetic divergence with a maximum value of 7.07% although the average value was low (0.97%). Lineage C included specimens exclusively from Bangladesh showing the lowest diversity with a maximum intraspecific genetic divergence of 4.06% and an average value of 1.50%. Genetic differentiation between lineages A and B ranged between 2.90% and 10.03% with an average value of 4.87% and with lineage C extended between 0.21% and 5.87% with an average of 2.00%. Lineages B and C showed high levels of genetic differentiation with the range of genetic divergence being between 3.68% and 8.81% with an average of 5.12%.

The MJ network based on the ITS-1 sequences revealed two genetically divergent lineages (A, B) within *C. oxystoma* plus one lineage (C) for *C. schultzei* (Figure 5b). Lineage A comprised all members of *C. oxystoma* from Thailand, Israel, Japan and India. Lineage B was represented by three sequences of *C. oxystoma* from Nigeria that were genetically different from lineage A by >9.49%. *Culicoides oxystoma* in lineage A was genetically different from *C. schultzei* by >16.67% and those of lineage B by >15.17%.
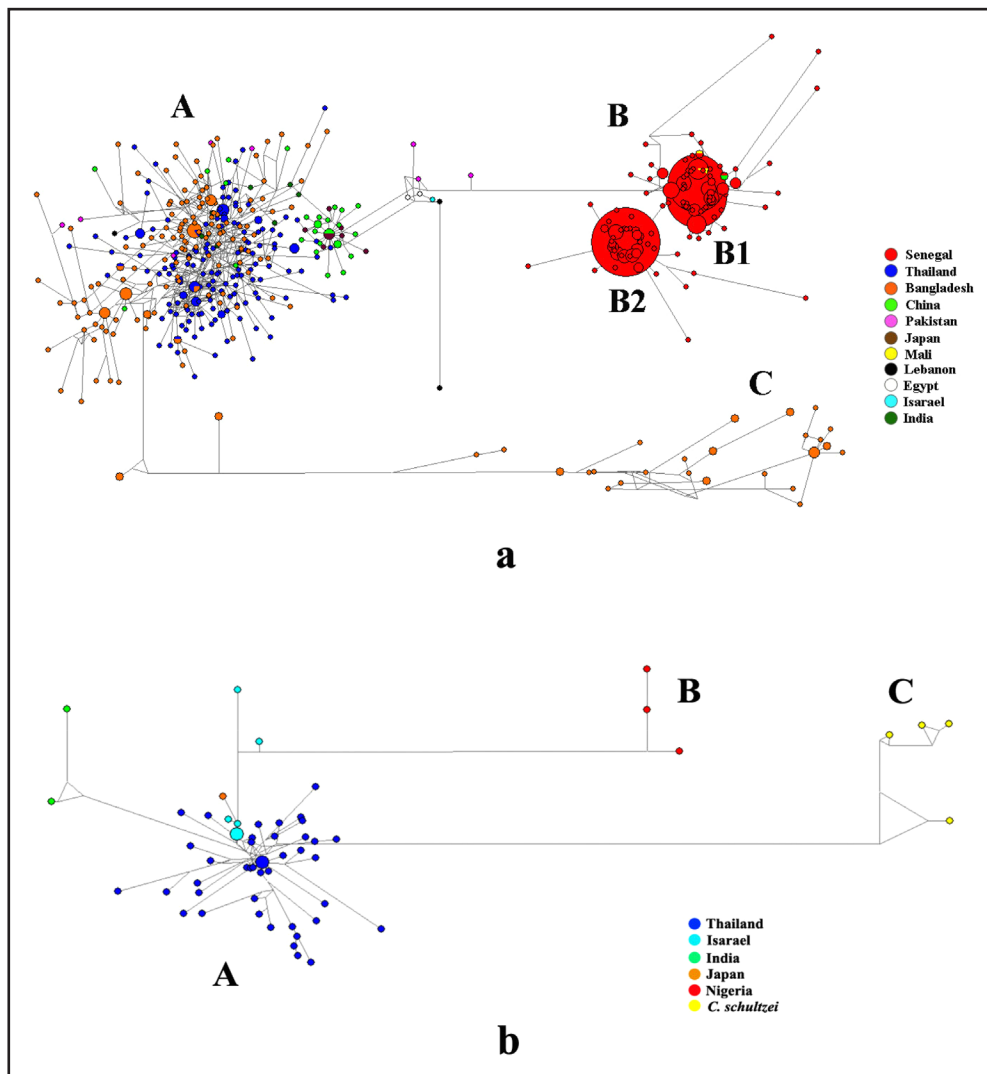

Figure 5. Median joining network of (a) 1,032 mitochondrial cytochrome c oxidase I (COI) sequences and (b) 56 ITS-1 sequences of *Culicoides oxystoma* and 4 sequences of *C. schultzei*. Each circle represents a haplotype and sizes are relative to the number of individuals sharing such haplotypes. Haplotypes are labelled according to the countries of the origins.

DISCUSSION

The level of intraspecific genetic divergence of *C. oxystoma* based on the COI gene sequences was relatively high compared to other *Culicoides* species in Thailand reported by Jomkumsing *et al.* (2021) and Gopurenko *et al.* (2022). The maximum intraspecific genetic divergence of *C. oxystoma* in Thailand based on COI sequences obtained in the present study (4.29%) was slightly higher than previously reported (3.45%) (Jomkumsing *et al.*, 2021), probably due to the larger sample size (161 vs 40) and wider geographic coverage. High level of genetic diversities were also found for the ITS-1 sequences with maximum intraspecific genetic divergence of 6.55% compared to 3.59% for *C. oxystoma* from Israel (Morag *et al.*, 2012).

Despite a high level of genetic divergence, we did not find a genetically divergent lineage in Thai specimens based on both COI and the ITS-1 sequences. Therefore, there were no indications of the possibility of cryptic species being present in our specimens. The high level of genetic diversity of *C. oxystoma* in Thailand suggest that this species has a large effective population size. This is potentially related to its relatively high abundance and being geographically widespread in Thailand (Pramual *et al.*, 2021). The larval stage of this species can utilize diverse aquatic and semiaquatic habitats such as stream margins, drains and ponds (Wirth & Hubert, 1989). Molecular analysis of blood meals revealed that *C. oxystoma* feeds on cattle (cow, water buffalo) (Jomkumsing *et al.*, 2021). Because cattle are abundant and widespread in Thailand, they potentially support the occurrence of a large number of *C. oxystoma*.

Despite a high level of genetic variation, no genetic structuring was observed within Thai populations of *C. oxystoma*. The population pairwise F_{ST} comparisons between 13 populations revealed no significant genetic differentiation although the range of geographic sampling covered more than 900 km from the northeast to the southern regions Thailand. The lack of genetic structuring based on pairwise F_{ST} agrees with the mitochondrial genealogical analysis. The MJ network indicated that there was no genetically divergent lineage or geographic clustering of the COI haplotypes within Thai specimens. Low or no genetic structuring of *Culicoides* biting midges has been reported in other geographically widely distributed species such as *C. obsoletus* (Pili *et al.*, 2010; Mignotte *et al.*, 2021) and *C. imicola* (Jacquet *et al.*, 2016). Previous study in a geographically co-distributed species in Thailand (*C. mahasarakhamense* Pramual, Jomkumsing, Piraonapicha, Jumpato), also found a low level of genetic structure (Pramual *et al.*, 2022). The low level of genetic differentiation between populations of *C. oxystoma* in Thailand could be a result of continuous distribution of the populations and the common movement of the host (i.e. cow, buffalo) *via* trading. Both cows and buffalo, which are the preferred blood meal sources of *C. oxystoma*, are commonly transported across the country during trading. The source and the destination market of cattle can be 700 km or more (Khengwa *et al.*, 2015). It is well recognized that the biting midge species can be co-transported with livestock (Purse *et al.*, 2015).

The global mtDNA genealogy revealed three genetically divergent lineages (A, B and C). These lineages showed a considerable level of genetic differentiation, particularly between lineage B and lineage C (3.68%–8.81%). Lineage B was also genetically distinct from lineage A with the minimum level of genetic divergence of 2.90%. Lineage A (the Indomalayan lineage) included specimens from Thailand and several countries covering South Asia (India, Bangladesh, Pakistan), Southeast Asia (Thailand), East Asia (China, Japan), the Middle East (Lebanon, Israel) and North Africa (Egypt). The genetically close relationship of *C. oxystoma* from these countries based on mitochondrial COI gene was also supported by the nuclear ITS-1 sequences. Because

the type locality of *C. oxystoma* is in India (Kolkata) (Wirth & Hubert, 1989), this lineage is most likely to represent the true *C. oxystoma*.

Lineage C was exclusively represented by specimens from Bangladesh. This lineage was genetically differentiated from lineage A (which is supposed to be the true *C. oxystoma*) by between 0.21% and 5.87% with an average of 2.00%. There are two possible explanations for the cryptic genetic divergence of lineage C. It is possible that lineage C is a relic of ancestral polymorphism. Alternatively, this genetically divergent lineage represents a different but morphologically similar species. Further investigation is requiring to test these hypotheses.

All specimens from Senegal were retrieved in lineage B with a considerable level of genetic differentiation compared to lineage A (2.90%–10.03%) and lineage C (3.68%–8.81%). Genetic differentiation of specimens identified as *C. oxystoma* from Senegal has been reported previously, leading to the suggestion that this species is possibly a species complex (Cornet *et al.*, 1994; Bakhoum *et al.*, 2013; Harrup *et al.*, 2016; Bakhoum *et al.*, 2018). Bakhoum *et al.* (2013) found that *C. oxystoma* from Senegal, although genetically similar, is still distinct from those of Japan, Australia and Israel with >4% genetic differentiation based on COI sequences. Similar levels of genetic differentiation (3.7%–5.8%) between Senegalese *C. oxystoma* and specimens from other countries have also recorded by Harrup *et al.* (2016) and more recently by Rot *et al.* (2020). Taken together, the results of the present study support those of previous findings that the so called '*C. oxystoma*' in Senegal is likely to be another biological species. Interestingly, in the present study, we found that based on shared haplotypes, this Senegalese *C. oxystoma* has also been found in nearby Mali and in a geographically much more distant region, China. This possibly indicates the long-distance dispersal of this species which might have been mediated by humans.

CONCLUSION

Population genetic structure and diversity analyses of *C. oxystoma* in Thailand revealed a relatively high level of genetic diversity compared to other species of biting midges in the country. This high level of genetic diversity in *C. oxystoma* agrees with reports from other geographic region that also reported similar levels. However, we found no genetic structure amongst populations of this species collected throughout the country. Long distance dispersal, possibly through human agency, the continuous distribution of suitable habitats and availability of the preferred host blood sources (i.e. cattle, buffalo) are factors promoting gene flow and thus reducing genetic differentiation. The high movement rate of *C. oxystoma* in Thailand indicates that there is a potential risk of spreading disease causing agents and that this species is potentially a competent vector for example for *Leishmania martiniquensis* and *L. orientalis* and BTV (Fujisawa *et al.*, 2021; Sunantaraporn *et al.*, 2021). The mitochondrial haplotype network revealed that populations in Thailand belong to the genetic lineage which is presumably the true *C. oxystoma*, which is present in North Africa, East, South and Southeast Asia.

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Conflict of interest
The authors declare no conflict of interest.

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