Heliyon 9 (2023) e13255

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Description of potential vectors of zoonotic filarial nematodes, *Brugia pahangi*, *Setaria digitata*, and *Setaria labiatopapillosa* in Thai mosquitoes

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ARTICLE INFO

Keywords:
Potential vector
Mosquitoes
Zoonotic filarial nematodes
Brugia pahangi
Setaria digitata
Setaria labiatopapillosa

ABSTRACT

Filariasis is classified as a vector-borne zoonotic disease caused by several filarial nematodes. The disease is widely distributed in tropical and subtropical regions. Understanding the relationship between mosquito vectors, filarial parasites, and vertebrate hosts is therefore essential for determining the probability of disease transmission and, correspondingly, developing effective strategies for prevention and control of diseases. In this study, we aimed to investigate the infection of zoonotic filarial nematodes in field-caught mosquitoes, observe the potential vectors of filaria parasites in Thailand using a molecular-based survey, conduct a study of host-parasite relationship, and propose possible coevolution of the parasites and their hosts. Mosquitoes were collected around cattle farms in Bangkok, Nakhon Si Thammarat, Ratchaburi, and Lampang provinces from May to December 2021 using a CDC Backpack aspirator for 20–30 minutes in each area (intra-, peri-, and wild environment). All mosquitoes were identified and morphologically dissected to demonstrate the live larvae of the filarial nematode. Furthermore, all samples were tested for filarial infections using PCR and sequencing. A total of 1,273 adult female mosquitoes consisted of five species: 37.78% *Culex quinquefasciatus*, 22.47% *Armigeres subalbatus*, 4.71% *Cx. tritaeniorhynchus*, 19.72% *Anopheles peditaeniatus*, and 15.32% *An. dirus*. Larvae of *Brugia pahangi* and *Setaria labiatopapillosa* were found in *Ar. subalbatus* and *An. dirus* mosquitoes, respectively. All

Abbreviations: COXI, Cytochrome c oxidase subunit I; *ITS1*, Internal transcribed spacer 1 gene; PCR, Polymerase chain reaction; VBDs, Vector-borne diseases.

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https://doi.org/10.1016/j.heliyon.2023.e13255
Received 22 July 2022; Received in revised form 16 January 2023; Accepted 24 January 2023
Available online 26 January 2023

mosquito samples were processed by PCR of *ITS1* and *COXI* genes for filaria nematode species identification. Both genes showed that *B. pahangi* was found in four mosquitoes of *Ar. subalbatus* from Nakhon Si Thammarat, *S. digitata* was detected in three samples of *An. peditaeniatus* from Lampang, and *S. labiatopapillosa* was detected in one of *An. dirus* from Ratchaburi. However, filarial nematodes were not found in all *Culex* species. This study infers that this is the first data regarding the circulation of *Setaria* parasites in *Anopheles* spp. from Thailand. The phylogenetic trees of the hosts and parasites are congruent. Moreover, the data could be used to develop more effective prevention and control strategies for zoonotic filarial nematodes before they spread in Thailand.

1. Introduction

Vector-borne diseases (VBDs) are transmitted by the bites of infected blood-sucking arthropods such as mosquitoes, ticks, sandflies, and black flies. The causative agents of the diseases include viruses, bacteria, and parasites [1]. Mosquito-borne diseases are among the most critical global public health problems, causing deaths in human, wildlife, and livestock and economic losses [2]. One of the parasitic infections transmitted by mosquitoes, filariasis, is a significant disease caused by filarial nematode worm infections. It is transmitted by various mosquito genera, including *Mansonia*, *Anopheles*, *Aedes*, *Culex*, *Armigeres*, and *Ochlerotatus* [3,4]. Many filarial genera, including *Wuchereria, Brugia, Dirofilaria*, *Onchocerca*, *Dipetalonema*, *Loaina*, and *Meningonema*, have been isolated from human [5]. Filarial worms have been discovered in the heart and lungs, the subcutaneous tissues, the eye, the lymphatic system, and the central nervous system [6]. On the other hand, wild and domestic animals have been reported in Thailand and worldwide with *Dirofilaria immitis*, *D. repens*, *Brugia pahangi*, and *Acanthocheilonema reconditum* [7–10]. In addition, the genus *Setaria* nematode consists of 43 species found in the abdominal cavity of artiodactyls in a wide range of animals, including cattle, sheep, equines, and pigs, and therefore are found around the world [11]. The disease caused by *Setaria* nematodes is called setariasis or setariosis. In South Korea, *Setaria digitata* was the most common cause of equine neurological ataxia [12]. Subhachalat et al. [13] reported the first case of *S. digitata* in cattle in Thailand, but clinical manifestations are unknown. However, the signs and symptoms of filariasis depend on where the adult and larval nematodes are found, the severity of the infection is related to parasite species, and the number of parasites present [14]. According to data from the Department of Disease Control, Ministry of Public Health, Thailand, from 2022, there were 7 cases (0.01 per 100,000 population) of filariasis in the northern, southern, central, and northeastern regions of Thailand [15]. Filariasis elimination and control strategies are focused on the mosquito vectors and their potential reservoirs. Several studies in Thailand revealed the prevalence of filarial nematodes in both humans and animals; especially, zoonotic species have increased attention in dogs, cats, and other domestic animals in different regions of Thailand [9,16–18]. However, the occurrence of other species of filarial parasites, such as *Setaria* spp., remains largely unknown. In addition, information on filariasis in terms of the relationship with their hosts, vectors, geographic distribution, and association with human or veterinary diseases is also essential. Therefore, the present study was to determine the infection of zoonotic filarial nematodes in field-caught mosquitoes and estimate the potential vector capacity of mosquitoes in various regions of Thailand using a molecular-based survey study of host-parasite interactions and coevolution. The information from studies of filarial species and mosquito species interactions are essential in understanding the risk of disease transmission and implementing appropriate mosquito management in endemic areas.

2. Materials and methods

2.1. Sample collection and preparation

A total of 1,273 adult female mosquitoes collected for this study were from four provinces: Bangkok (central), Nakhon Si Thammarat (southern), Ratchaburi (western), and Lampang (northern) provinces, Thailand during 20–30 minutes in each area, including intra- and peridomestic environments from May to December 2021 (Table 1) using CDC Backpack aspirator (Bioquip, USA). All

Table 1
The number of adult female mosquito species collected from various regions of Thailand.

Province	Region	Mosquito species	No. Mosquitoes
Bangkok	central	*Armigeres subalbatus*	106
		Culex quinquefasciatus	201
		Cx. tritaeniorhynchus	60
Nakhon Si Thammarat	southern	*Ar. subalbatus*	180
		Cx. quinquefasciatus	150
Ratchaburi	western	*Anopheles dirus*	110
		An. peditaeniatus	98
Lampang	northern	*Cx. quinquefasciatus*	130
		An. dirus	85
		An. peditaeniatus	153
Total			**1273**

mosquitoes were differentiated based on morphological identification according to their sex and species. Moreover, each female mosquito was dissected under a stereomicroscope on a sterilized slide using sterile needles. The slide was demonstrated for the live larvae of filarial nematodes in all female mosquitoes under a light microscope (Olympus, Tokyo, Japan). The mosquito carcasses of each female specimen were transferred to sterile 1.5 ml Eppendorf tubes with a lysis buffer for the detection of filarial nematode species by polymerase chain reaction (PCR) of the internal transcribed spacer 1 (*ITS1*) and the cytochrome *c* oxidase subunit I (*COXI*) genes, which are both suitable for use as molecular markers for a high degree of genetic variation of filarial nematodes.

2.2. DNA extraction

According to the manufacturer's instructions, DNA was extracted from the individual mosquito samples using the Invisorb Spin Tissue Mini Kit (Berlin, Germany). Extracted DNA was eluted in 50 μl of elution buffer. Genomic DNA samples were used for filarial nematode species detection immediately, and the rest of the samples were stored at −80 °C for long-term storage.

2.3. Molecular identification of filarial nematodes

The DNA samples were amplified for partial *ITS1* and *COXI* genes. The ITS1 region was PCR-amplified using forward primer ITS1-F (5′GGTGAACCTGCGGAAGGATC3′) and reverse primer ITS1-R (5′CTCAATGCGTCTGCAATTCGC3′), and this was performed in 25 μl of reaction mixture containing 5 μl of DNA template, 2.5 μl of 10× buffer, 25 mM of $MgCl_2$, 2 mM of dNTPs, 10 μM of each primer, and 1 unit of *Taq* DNA polymerase (Thermo Fisher Scientific, USA). The PCR conditions were 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s, and 72 °C for 10 min [19]. The *COXI*-PCR was performed by employing the two primers, COI-int-F (5′TGATTGGTGGTTT TGGTAA3′) and COI-int-R (5′ATAAGTACGAGTATCAATATC3′) [20]. The reaction was carried out in a total volume of 25 μl, containing 2.5 μl of 10× PCR Buffer, 25 mM of $MgCl_2$, 2.5 mM of each dNTP, 10 mM of each primer, and 1 unit of *Taq* DNA polymerase (Thermo Fisher Scientific, USA), and 5 μl of DNA template. The reaction mixture was incubated at 94 °C for 3 min, 40 cycles of 94 °C for 45 s, 52 °C for 45 s, 72 °C for 90 s, and 72 °C for 7 min. The positive controls used *D. immitis* and *B. malayi* DNA, whereas double-distilled water was used as a negative control. The amplified PCR products were electrophoresed through a 1.5% agarose gel, stained with ethidium bromide, and then visualized with Quantity One Quantification Analysis Software Version 4.5.2, Gel Doc EQ System (Bio-Rad, USA).

2.4. Molecular identification of mosquito samples

The mosquito identifications were confirmed by analyzing the partial mitochondrial cytochrome C oxidase subunit I (*COXI*) gene. The *COXI* gene was amplified using a forward primer (C1-J-1718-F; 5′CCCGGTAAAATTAAAATATAAACTTC3′) and reverse primer (C1–N-2191-R; GATTTGGAAATTGATTAGTTCCTT). The PCR reaction and amplification profiles followed the protocol previously described in Simon et al. [21]. Plasmid DNA containing the *COXI* gene of *Ae. aegypti* was used as the positive control, and sterilized distilled water was used as a negative control.

2.5. Cloning and sequencing

The positive PCR amplicons were cloned into the pGEM-T Easy Vector (Promega, USA), and the recombinant plasmids were used to transform a competent *Escherichia coli* DH5α strain for screening the bacterial transformants using the blue-white colony system. The plasmid DNA was extracted using the Invisorb Spin Plasmid Mini kit (Berlin, Germany) following the manufacturer's instructions. The purified plasmids were then used for direct DNA sequencing by a commercial sequencing company (Macrogen, South Korea).

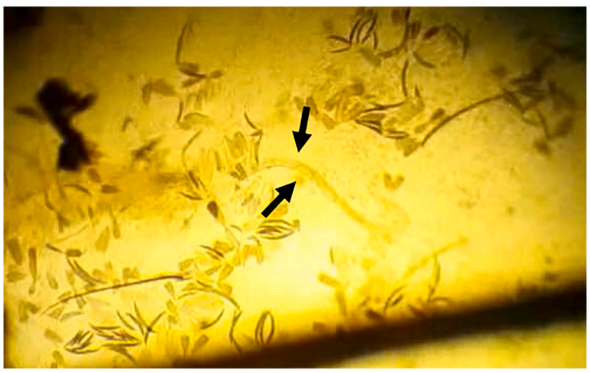

Fig. 1. Live filarial larvae in *Ar. subalbatus* adult female mosquito under a light microscope and observe the movement in the attached video (Supplementary data).

2.6. Sequence and phylogenetic analysis

The genome sequences were assembled using multiple alignments using fast Fourier transform (MAFFT) version 7 (https://mafft.cbrc.jp/alignment/server/) [22]. The phylogenetic trees were constructed using the maximum-likelihood method with IQ-TREE on the IQ-TREE web server (http://iqtree.cibiv.univie.ac.at/) with 1,000 ultrafast bootstrap replicates. The best-fit substitution model was found using the auto function on the IQ-TREE web server [23]. Eventually, the phylogenetic tree was generated using FigTree v.1.4.4 software (http://tree.bio.ed.ac.uk/software/figtree/). The visualization of relationship between filarial nematode parasites and their mosquito hosts was created based on phylogenetic analysis as described above and customized by the interactive tree of life webserver (iTOL) version 6.5.7 (https://itol.embl.de), and Adobe Illustrator 2020.

3. Results

Based on the composition of the species of mosquito caught, 37.78% *Cx. quinquefasciatus*, 22.47% *Ar. subalbatus*, 4.71% *Cx. tritaeniorhynchus*, 19.72% *An. peditaeniatus*, and 15.32% *An. dirus* were collected from various regions of Thailand. Alive filarial larvae from mosquitoes can be found in dissection of *Ar. subalbatus* and *An. dirus* from Nakhon Si Thammarat and Ratchaburi, respectively (Fig. 1 and video in Supplementary data). Molecular diagnostic methods confirmed parasite DNA amplification by *COXI*-PCR and *ITS1*-PCR in all samples. The 8/1,273 (0.63%) mosquito samples included four *Ar. subalbatus*, one *An. dirus*, and three *An. peditaeniatus* samples were positive for both gene fragments (Table 2). The *COXI* nucleotide sequences of all filarial nematodes in *Ar. subalbatus* showed 100% homology with reference *B. pahangi* sequences in the dog from Thailand (Accession no. MK250722) and female *Ar. subalbatus* from Malaysia (Accession no. DQ977746) available on GenBank, and the *ITS1* sequences showed 99.41–100.00% sequence identity with *B. pahangi* in dog from Thailand (Accession no. MK250763 and MK250800). One positive PCR from *An. dirus*, the *COXI* sequence showed 93.02% similarity with *S. labiatopapillosa* in sheep from China (Accession no. NC044071). In contrast, a BLAST result of the *ITS1* sequence demonstrated a 78.99% match with *Setaria yehi* in white-tailed deer (*Odocoileus virginianus*) from the USA (Accession no. KU757075). Additionally, three *COXI*-PCR positives from *An. peditaeniatus* shared 99.71% nucleotide identity with *S. digitata* in buffalo from China, and *ITS1* sequences showed a 79.35% match with *S. yehi* in white-tailed deer (*O. virginianus*) from the USA (Accession no. KU757075). However, the result of filarial nematodes in *Culex* spp. was not found in all samples. The genetic relationship between filarial nematodes obtained in this study and other filarial nematodes from geographically diverse areas was determined by a phylogenetic tree based on *COXI* and *ITS1* genes. The results revealed that all eight *COXI* (Fig. 2A) and eight *ITS1* (Fig. 2B) sequences were clearly classified under *B. pahangi*, *S. labiatopapillosa*, and *S. digitata*. The nucleotide sequences of two genes from this study were submitted in the GenBank database. The eight *COXI* (Accession no. ON512447-ON512454) and eight *ITS1* (Accession no. ON512455-ON512462) sequences from four *B. pahangi*, one sequence of *S. labiatopapillosa*, and three sequences of *S. digitata* were deposited in Genbank. For the genetic variability of mosquito vectors, a partial nucleotide sequence of 524 bp from the *COXI* gene was sequenced in all filarial infected mosquitoes and some uninfected mosquitoes in this study. The phylogenetic tree showed the sequences of all filarial infected mosquitoes, and some uninfected mosquitoes were strongly grouped and classified as *Ar. subalbatus*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, *An. peditaeniatus*, and *An. dirus* when compared to published sequences available in the GenBank database. The findings demonstrate that *Anopheles* mosquitoes segregate into distinct monophyletic groupings from *Culex* spp., *Armigeres* spp., *Aedes* spp., and *Mansonia* spp. The *Armigeres* spp. Group is a sister clade to the entire group of *Aedes* mosquitoes. Moreover, the genetic diversity of filarial-infected mosquito populations did not differ from uninfected mosquitoes (Fig. 3). Therefore, the association between the filarial worm and the mosquito has attracted great interest. We constructed two phylogenetic trees to analyze the parasites and hosts that occupy corresponding positions. Our results demonstrated that the two phylogenies are linked to host-parasite interactions. We found several instances in which a single mosquito species acts as a host for two or more related filaria nematode species. We observe five species of *Setaria* parasite (*S. cervi*, *S. digitata*, *S. equina*, *S. tundra*, and *S. labiatopapillosa*) related to *Anopheles* spp., *Culex* spp., *Armigeres* spp., and *Aedes* spp.. In this study, *S. digitata* was associated with *Anopheles* spp., *Culex* spp., and *Aedes* spp., whereas *S. labiatopapillosa* was linked with *Anopheles* spp., *Culex* spp., *Armigeres* spp., and *Aedes* spp.. Human act as accidental hosts for the *S. digitata* and *S. labiatopapillosa* parasites. For *B. pahangi*, it has also been connected to *Armigeres* spp. and *Mansonia* spp.. *Aedes* mosquitos have been related to the parasites *Setaria*, *Brugia*, and *Wuchereria* (Fig. 4).

Table 2
Details on the detection of mosquitoes and filarial worms.

Province	Region	Code	Mosquito species (Morphology and molecular identification)	Alive filarial larvae	Molecular detection of parasites (*COXI* and *ITS1*)
Nakhon Si Thammarat	southern	Ar1	*Ar. subalbatus*	Found	*B. pahangi*
		Ar2	*Ar. subalbatus*	Found	*B. pahangi*
		Ar3	*Ar. subalbatus*	Not found	*B. pahangi*
		Ar4	*Ar. subalbatus*	Not found	*B. pahangi*
Ratchaburi	western	An1	*An. dirus*	Found	*S. labiatopapillosa*
Lampang	northern	An9	*An. peditaeniatus*	Not found	*S. digitata*
		An10	*An. peditaeniatus*	Not found	*S. digitata*
		An13	*An. peditaeniatus*	Not found	*S. digitata*

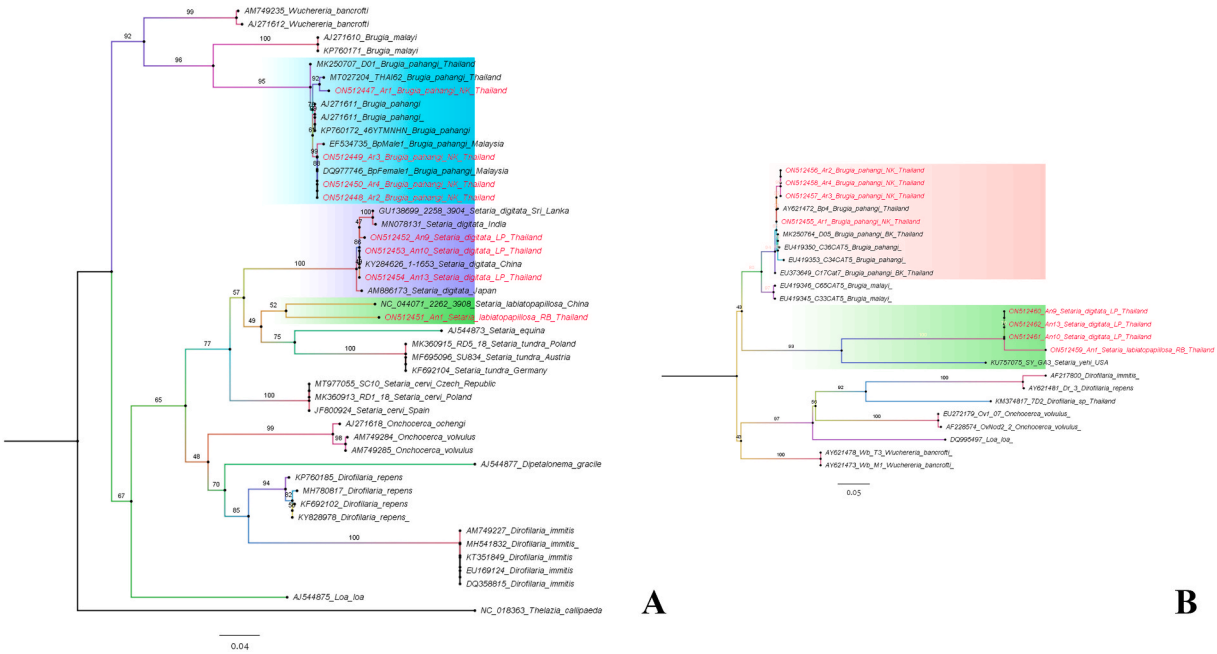

Fig. 2. Phylogenetic analysis of the partial *COXI* (633–689 nt) (**A**) and *ITS1* (511–631 nt) (**B**) filarial nematode's sequences and reference sequences. The phylogenetic tree was constructed with IQ-TREE using the maximum-likelihood method with 1000 ultrafast bootstrap replicates. The best-fit substitution model was found using the auto function on the IQ-TREE web server. The sequences from this study are indicated in red.

4. Discussion

Pathogen, vector, and host factors are important in transmitting vector-borne diseases [24]. Basic knowledge of relationship against the disease vectors including vector populations, competency, and parasite-host ecology interaction would increase the understanding of disease transmission and therefore it could be used for prevention and control of the diseases. The present study described the infection of zoonotic filarial nematodes in field-caught mosquitoes, investigated the potential filaria vectors in Thailand using a molecular-based survey, and studied host-parasite relationships. Our results demonstrated that *An. dirus*, *An. peditaeniatus*, and *Ar. subalbatus* were the natural vectors for *S. labiatopapillosa*, *S. digitata*, and *B. pahangi*, respectively. To our knowledge, this is the first report of *Setaria* parasite circulation in *Anopheles* spp. from Thailand. *Setaria* parasites are a genus of filarial roundworms that infect swine, camels, cattle, horses, and other domestic mammals [25]. Adult *Setaria* worms found in the peritoneal cavity do not cause any major damage to the host. Nevertheless, they cause harmful effects in unusual locations, reaching the central nervous system, migrating along nerves, sometimes to the eye, occasionally to the fetus of pregnant animals, causing severe injury, paralysis, and death within days [26]. *S. digitata*, *S. marshalli*, *S. cervi*, and *S. labiatopapillosa* are *Setaria* parasites commonly found in cattle [27,28]. However, Subhachalat et al. [13] showed the first report of *S. digitata* from cattle, which were bred and housed in Nonthaburi Province in Thailand, but the clinical symptoms remain unknown. Recently, Ngasaman et al. [29] revealed that microfilaria of *Setaria* spp. (2.68%) were found in bullfighting cattle in the southern part of Thailand. Therefore, disease control and prevention in cattle, as reservoirs of *Setaria* parasites, must be considered along with vector control in Thailand. The data from this study supported that mosquito vectors *An. dirus*, *An. peditaeniatus* are potential vectors of *S. labiatopapillosa* and *S. digitata*, respectively, whereas filarial nematodes were not detected in *Culex* mosquitoes. However, the first report of *Cx. quinquefasciatus* demonstrates vector competence for *S. digitata* in Taiwan [30]. *Ae. claviger* and *Ae. maculipennis* appear to serve as vectors of *S. labiatopapillosa* [31]. In addition, other *Aedes* spp. [32], *Anopheles* spp. [33], and horn flies [34] are known insect vectors for *Setaria* spp. Currently, *B. pahangi* can be observed in *Ar. subalbatus*. *B. pahangi* can be transmitted to human by reservoir animals such as cats and dogs [35]. In Thailand and other countries in Southeast Asia, the principal vectors of *B. malayi* are *Mansonia* mosquitoes including *Ma. uniformis*, *Ma. indiana*, *Ma. annulifera*, and *Ma. onneae* [36,37]. *Ar. subalbatus* is the natural vector for zoonotic *B. pahangi* and can also transmit the disease to human [38]. Recently, Intarapuk and Bhumiratana [17] investigated *Ar. subalbatus* is a vector of zoonotic *B. pahangi* in Suratthani, Southern Thailand, where Thai children have been infected with zoonotic *B. pahangi*. From the aforementioned discussion, it can provide information about circulating pathogens and their vectors. In addition, we also have the nucleotide sequences of filaria nematodes and mosquitoes based on the molecular survey. The two phylogenetic trees were constructed to analyze the coevolution of hosts and parasites. Comparing the phylogenies of filariae and mosquitoes revealed significant differences in the co-phylogenies of each parasite group. At the general level, we continue to observe a strong relationship between the parasite lineage and the host group, as indicated by largely congruent phylogenies. *Anopheles* spp., *Culex* spp., *Armigeres* spp., and *Aedes* spp. are almost all found to carry the *Setaria* parasites. *S. digitata* was linked with *Anopheles* spp., *Culex* spp., and *Aedes* spp., whereas *S. labiatopapillosa* was linked with

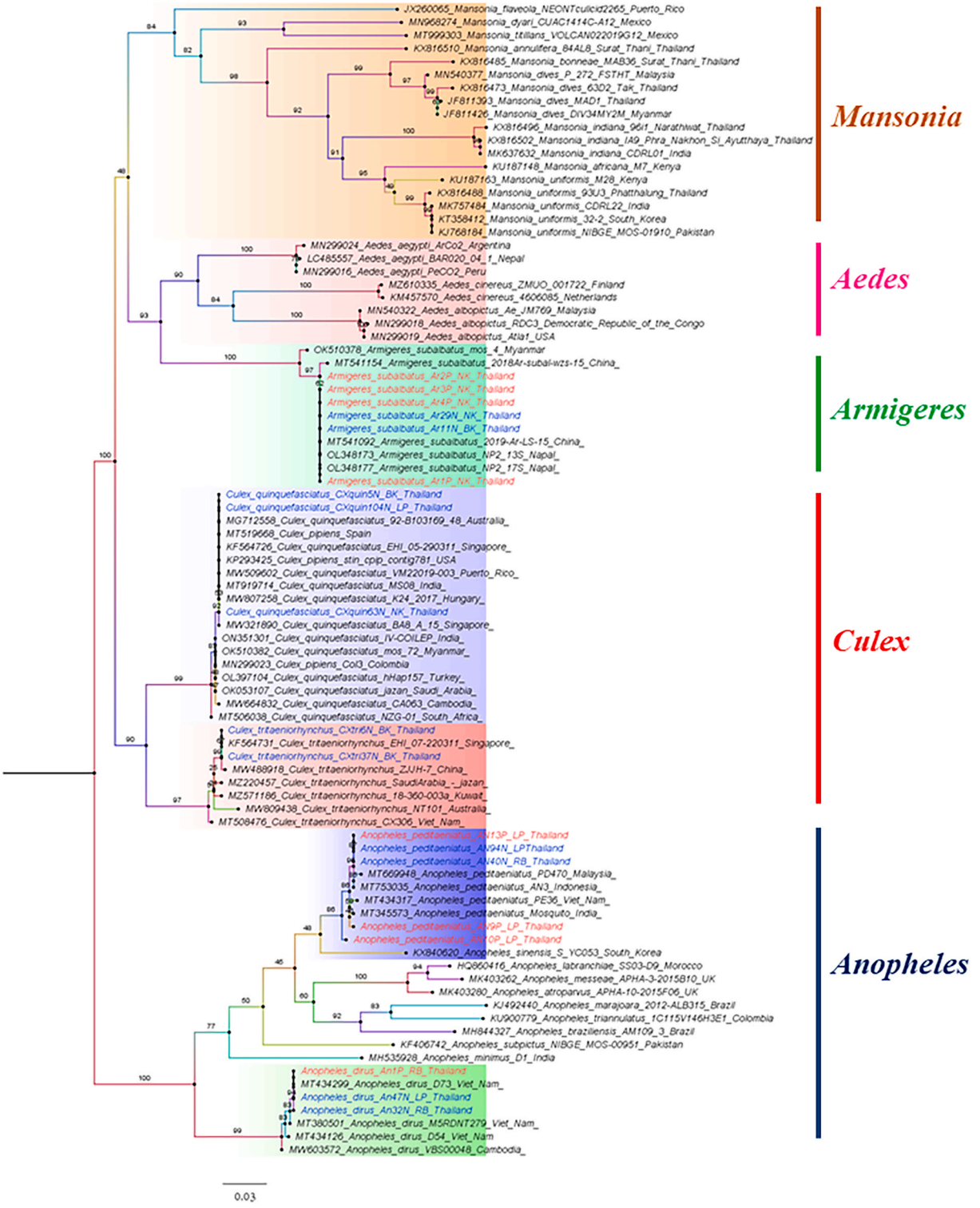

Fig. 3. Phylogenetic tree based on partial nucleotide sequences of the *COXI* gene of all filarial-infected mosquitoes and some uninfected mosquitoes, compared to reference sequences available in the GenBank database. The sequences from filarial infected mosquitoes are indicated with a red color, whereas uninfected mosquitoes are blue.

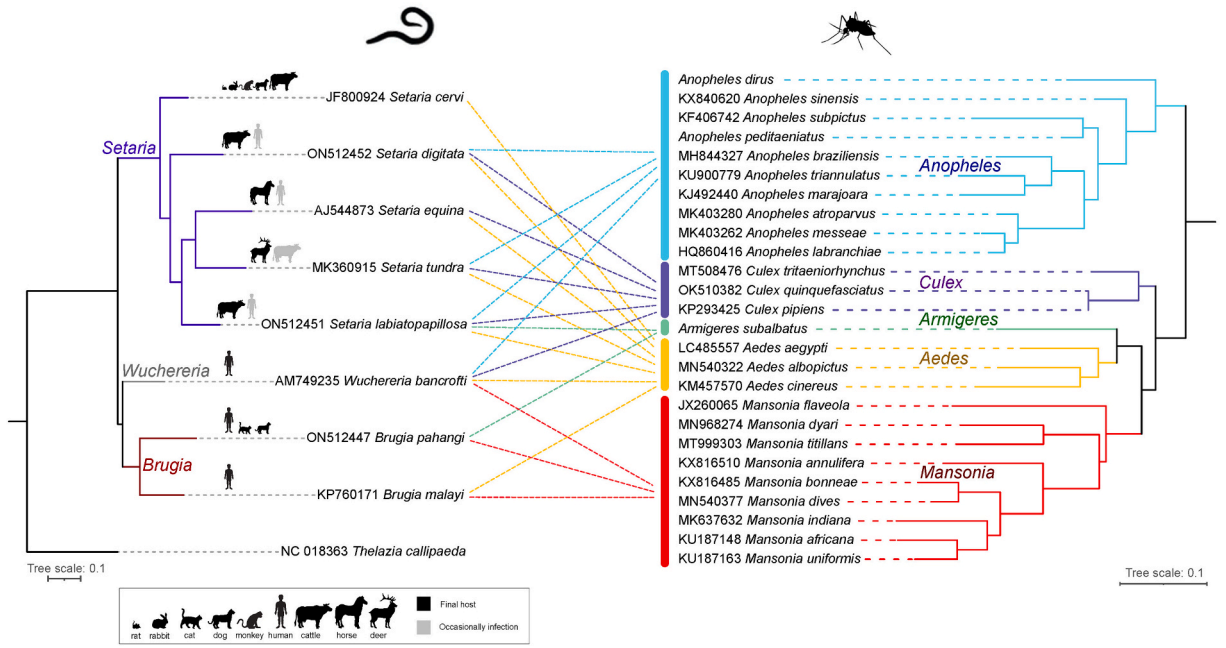

Fig. 4. Two phylogenetic trees of filarial nematode parasites and their mosquito hosts. Dashed lines connect taxa, and colors indicate specific mosquito host and filarial nematode parasite relationship based on the previous and current findings. Human and animal silhouettes were obtained from http://www.freepik.com and http://www.vecteezy.com.

Anopheles spp., *Culex* spp., *Armigeres* spp., and *Aedes* spp. Human serve as accidental hosts for the parasites *S. digitata* and *S. labiatopapillosa*. The *B. pahangi* has also been associated with mosquitoes such as *Armigeres* spp. and *Mansonia* spp. *Setaria*, *Brugia*, and *Wuchereria* parasites have all been related to *Aedes* mosquitoes. The results showed the diversity of filaria nematode species in one mosquito and provided information about filaria nematode associations with their hosts and their adaptation in mosquitoes. Additionally, vector immunity may have been co-adapted during co-evolution with the parasite. In *Ae. albopictus*, *S. digitata* did not develop the infective stage (L3) but succeeded in another *Ae. aegypti* local strain. As a result, *Ae. albopictus* could be a refractory vector and the microfilariae in the hemocoel were melanized and encapsulated [39]. The present study has suggested the lack of basic knowledge of filarial larvae in their vectors in Thailand. Moreover, regarding the study's limitations, the number of collected mosquitoes was relatively low. For example, only *Anopheles* mosquitoes were found in Ratchaburi province, with no *Culex* or *Armigeres* mosquitoes. This could be attributable to the behavior of these mosquitos and the mosquito species' breeding habitats. Water source and water quality have an impact on mosquito distribution [40]. The most common *Anopheles* mosquito habitats suitable in Ratchaburi were natural and clear water bodies and were abundant in permanent and full sunlight habitats [41]. However, the *Culex* mosquito has been reported to breed in polluted waters and sites with foul smells [42]. *Armigeres* mosquitoes are found in natural small container habitats where waters are extremely foul or with high organic content [43]. This why the number of mosquitoes in some species collected in this study was low. Therefore, extensive surveys to demonstrate its presence in its natural vectors or hosts may benefit from demonstrating early signals of its circulation before the emergence of diseases. For future studies, we would like to know if the mosquito-associated parasites can be (1) mosquito-borne parasites, which can replicate in mosquitoes and infect the vertebrates, and (2) mosquito-specific parasites, which can infect mosquitoes but do not infect vertebrates. The information could also help predict probable disease development in animals or susceptible human and determine the most effective diagnostics and strategies to prevent disease transmission.

5. Conclusion

Our results indicated that mosquitos, *An. dirus*, *An. peditaeniatus*, and *Ar. subalbatus* are competent vectors of *S. labiatopapillosa*, *S. digitata*, and *B. pahangi*, respectively. Furthermore, the two phylogenies clarify the interaction between vector genera and helminth transmission to design a specific vector-borne control program in Thailand. The data could potentially be used to predict and prevent outbreaks.

Ethics approval

The study protocol was approved by the animal research ethics committee of Chulalongkorn University Animal Care and Use Protocol (CU-ACUP), Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, based on the Ethics of Animal Experimentation of the National Research Council of Thailand. The certificate of approval number is 018/2565.

Author contribution statement

Conceptualization; A. Phumee, P. Siriyasatien.Data curation; A. Phumee, P. Intayot, V. Sawaswong, K. Preativatanyou, S. Wacharapluesadee, P. Siriyasatien. Formal analysis; A. Phumee, P. Intayot, V. Sawaswong, P. Siriyasatien. Funding acquisition; A. Phumee, P. Siriyasatien. Investigation; A. Phumee, P. Intayot, V. Sawaswong, K. Preativatanyou, S. Wacharapluesadee, R. Boonserm, S. Sor-suwan, P. Ayuyoe, A. Cantos-Barreda, P. Siriyasatien. Methodology; A. Phumee, P. Intayot, V. Sawaswong, R. Boonserm, S. Sor-suwan, P. Ayuyoe. Project administration; A. Phumee, P. Siriyasatien. Resources; A. Phumee, P. Siriyasatien. Writing-original draft; A. Phumee. Wrigint-review & editing; A. Phumee, Intayot, V. Sawaswong, K. Preativatanyou, S. Wacharapluesadee, R. Boonserm, S. Sor-suwan, P. Ayuyoe, A. Cantos-Barreda, P. Siriyasatien.

Funding statement

Prof. Dr. Padet Siriyasatien was supported by National Research Council of Thailand [NRCT5-RSA63001-03].

Asst. Prof. Dr. Atchara Phumee was supported by Office of the Permanent Secretary, Ministry of Higher Education, Science, Research and Innovation (Grant No. RGNS 64–208).

Declaration of interest's statement

The authors declare no competing interests.

Additional information

The nucleotide sequences from this study have been deposited in GenBank under the accession number ON512447-ON512462.

Acknowledgments

We would like to thank the Office of the Permanent Secretary, Ministry of Higher Education, Science, Research and Innovation (Grant No. RGNS 64–208), National Research Council of Thailand (Grant No. NRCT5-RSA63001-03), the Program Management Unit for Human Resources & Institutional Development, Research and Innovation-CU; Research Grant on Fundamental Fund: FF65 (Grant No. CUFRB65_hea(20)_027_30_08), and NIH/NIAID/CREID/07-049-7012-52338.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e13255.

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