



RESEARCH ARTICLE

Molecular detection and *cox1*-based phylogenetic analysis of *Strongyloides stercoralis* in fresh vegetables from lower northern Thailand

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ABSTRACT

Strongyloides stercoralis is an intestinal nematode with worldwide distribution, including Thailand, and contamination of vegetables poses a potential public health risk. Previous studies largely relied on light microscopy, often reporting nematode larvae without species identification. This study investigated the presence of *S. stercoralis* in fresh vegetables from the lower northern region of Thailand using both morphological and molecular methods, including *cox1*-based phylogenetic analysis. A total of 90 fresh vegetable samples were collected from farms across three provinces. In each province, 10 vegetable types; Chinese morning glory, lettuce, dill, green onion, celery, Chinese chives, Mustard green, coriander, gotu kola, and Chinese mustard were collected. Detection of *S. stercoralis* was performed by examining morphological characteristics under a microscope using the sedimentation technique. Molecular detection was carried out via PCR targeting the *cox1* gene, followed by sequencing and phylogenetic analysis. Of 90 samples, 47.78% were positive, with the highest contamination found in green onion (100%), lettuce (88.89%), and Chinese morning glory (66.67%). Molecular detection by PCR targeting the *cox1* gene (primers StrCoxAfrF/StrCoxAfrR) confirmed seven positive samples (23.33%) from four vegetable types: Chinese morning glory, lettuce, green onion, and Mustard green. Sequencing showed 99–100% identity with *S. stercoralis*, and phylogenetic analysis using the Neighbor-Joining method on the seven positive samples from three provinces revealed genetic affinity with strains from neighboring countries. These findings underscore the need for comprehensive public health strategies for surveillance, treatment, and prevention of *S. stercoralis* transmission through contaminated vegetables.

Keywords: Molecular; phylogenetic analysis; *Strongyloides stercoralis*; vegetables; lower northern part of Thailand.

INTRODUCTION

Strongyloides stercoralis is an intestinal roundworm that can be found worldwide, predominantly in tropical and subtropical regions (Buonfrate *et al.*, 2020). Infected individuals are often asymptomatic; however, severe and life-threatening manifestations may occur in immunocompromised patients. The parasite can cause recurrent infections through autoinfection, leading to severe disease (Chueansuwan & Chueansuwan, 2022). In such cases, hyperinfection may develop, particularly in individuals with compromised immunity, such as organ transplant recipients or patients receiving corticosteroids or immunosuppressive therapy. Hyperinfection reflects an acceleration of the normal life cycle of *S. stercoralis*, resulting in an overwhelming parasitic burden in the body, including the skin, intestines, and lungs, which can be fatal. This represents the most critical and severe manifestation of strongyloidiasis (Kassalik & Mönkemüller, 2011; Martviset, 2019). Contamination of vegetables with *S. stercoralis* can occur due to its free-living stages in the soil, from irrigation water, or from fertilizers derived from human or animal feces. Contamination may also happen

during harvesting, transportation, or marketing. The life cycle of *Strongyloides* spp. includes both parasitic and free-living stages in the soil. Transmission occurs primarily through skin penetration, while ingestion plays a minor role (Yeh *et al.*, 2023). Studies have shown that *S. stercoralis* can be transmitted from person to person through contact with bodily secretions (Czachor & Jonas, 2000). In agricultural settings, activities such as planting vegetables without gloves or using animal manure as fertilizer may increase the risk of infection. Reports from African markets indicate that fresh vegetables were contaminated with *S. stercoralis* (Zeynudin *et al.*, 2024). In Malaysia, studies on soil- and water-transmitted helminths found infective larvae of *S. stercoralis* in irrigation water used to wash vegetables such as spinach, indicating that vegetable vendors and food handlers are high-risk groups (Zeehaida *et al.*, 2011). Transmission can also occur through ingestion of water or food contaminated with the infective filariform larvae (Chueansuwan & Chueansuwan, 2022). In Titagarh, West Bengal, India, parasite contamination in vegetables was reported at 44.2% (Kudah *et al.*, 2018). Similarly, in Ethiopia, contamination of fresh vegetables was found in 63.8% of carrot samples and 63.1% of lettuce samples

(Zeynudin et al., 2024). In Southeast Asia, a study in Hanoi, Vietnam reported that 26% of vegetable were contaminated. (Uga et al., 2009). In Thailand, studies using the sedimentation technique have reported the prevalence of *S. stercoralis* ranging from 6.25% to 57% (Seawphukeaw et al., 2023; Pidsaya et al., 2025). Additionally, unidentified nematode larvae have been reported in 38.3–46.6% of samples (Krainara et al., 2022; Niamnuy et al., 2022). Previous investigations primarily relied on morphological identification under light microscopy, frequently reporting nematode larvae without species-level identification (Krainara et al., 2022; Niamnuy et al., 2022; Seawphukeaw et al., 2023; Gunathilaka et al., 2025). However, molecular studies of nematode larvae contaminating vegetables, particularly *S. stercoralis*, which can cause severe disease, remain limited. Therefore, this study aimed to detect *S. stercoralis* using both light microscopy and molecular techniques, including *cox1*-based phylogenetic analysis, in ten types of fresh vegetables collected from farms in the lower northern region of Thailand: Chinese morning glory, lettuce, dill, green onion, celery, Chinese chives, Mustard green, coriander, gotu kola, and Chinese mustard.

MATERIALS AND METHODS

Study sites

The study was conducted in three areas in the lower northern region of Thailand, as follows: Ban Dong Subdistrict, Chat Trakan District, Phitsanulok Province; Wang Bua Subdistrict, Khlong Khlung District, Kamphaeng Phet Province; and Phai Tha Pho Subdistrict, Pho Thap Chang District, Phichit Province (Figure 1).

Samples collections

A total of 90 fresh vegetable samples were collected from farms across three provinces of the lower northern region of Thailand. In each province, 10 types of vegetables were sampled on three occasions within a similar timeframe, resulting in 30 samples per province. The ten types of vegetables included Chinese morning glory (*Ipomoea aquatica*), lettuce (*Lactuca sativa*), dill (*Anethum graveolens*), green onion (*Allium fistulosum*), celery (*Apium graveolent*), Chinese chives (*Allium tuberosum*), Mustard green (*Brassica juncea*), coriander (*Eryngium foetidum*) Asiatic pennywort/ Gotu kola (*Centella asiatica*) and Chinese mustard/Pak choi (*Brassica rapa* subsp. *chinensis*) (Figure 2). From the farms, each type of

vegetable was collected in plastic bags, labeled with the vegetable name, collection date, and location, and transported for examination using the basic Sedimentation technique and the Polymerase Chain Reaction (PCR) technique at the Faculty of Medical Science Laboratory, Naresuan University, Phitsanulok Province.

Sedimentation technique

Fresh vegetable samples, including stems and leaves, were collected without prior washing. Roots were removed from vegetables that had them. A total of 100 g of each sample was cut into small pieces and placed in a 500 mL Erlenmeyer flask. Distilled water was added to completely cover the plant material. The mixture was then shaken on a shaker at 160 rpm for 1 hour to allow sediment and potential parasitic stages to detach from the vegetable surfaces. This method was modified from the procedure described by Boonyaksem et al.,

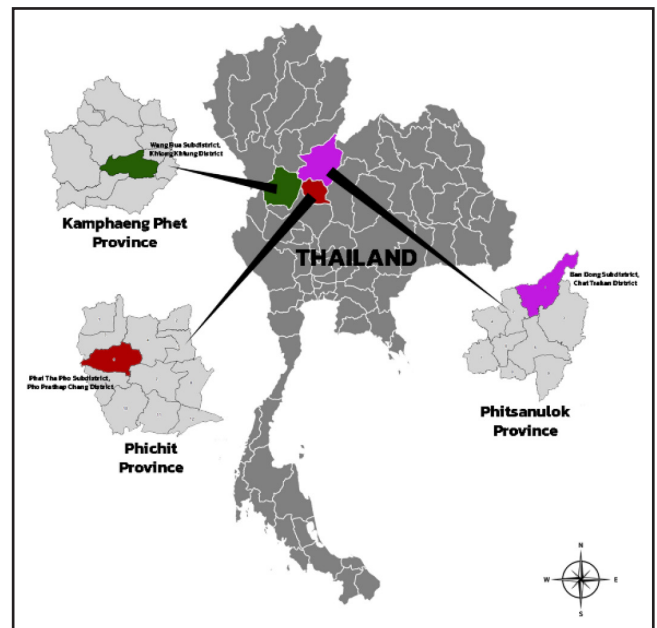


Figure 1. Geographical map depicts the study area across three provinces situated within the lower northern region of Thailand.

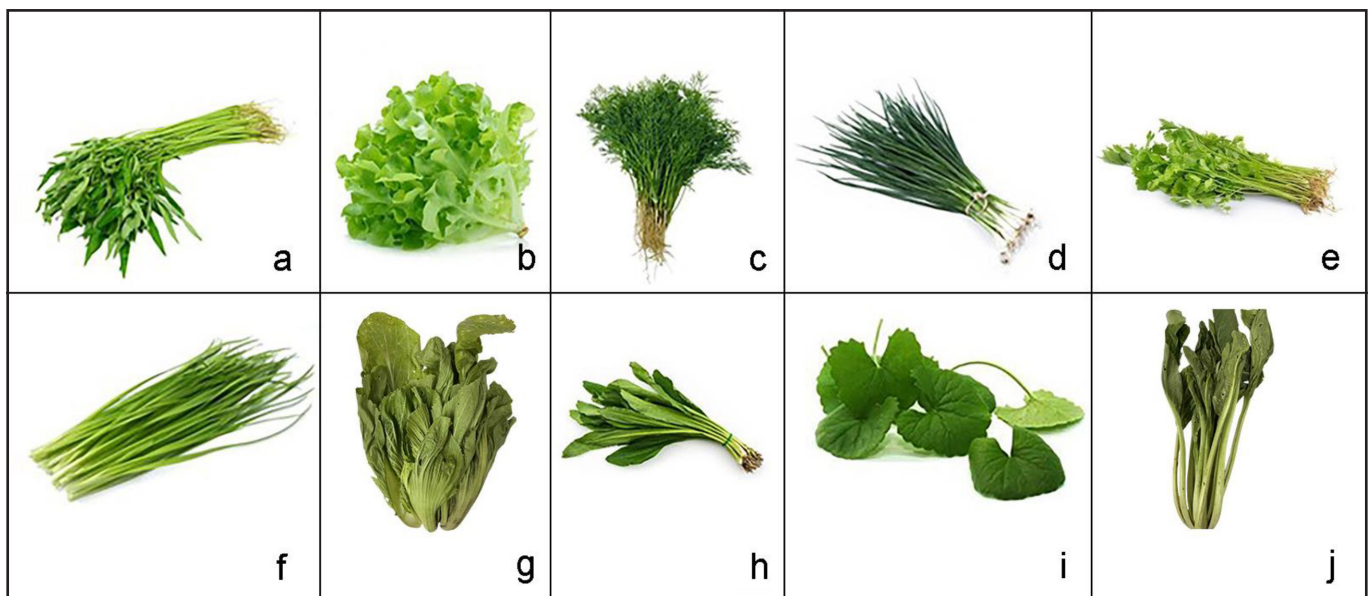


Figure 2. The picture of fresh vegetables sample a. Chinese morning glory (*I. aquatica*), b. lettuce (*L. sativa*), c. dill (*A. graveolens*), d. green onion (*A. fistulosum*), e. celery (*A. graveolent*), f. Chinese chives (*A. tuberosum*), g. Mustard green (*B. juncea*), h. coriander (*E. foetidum*) i. Asiatic pennywort/Gotu kola (*C. asiatica*) and j. Chinese mustard/Pak choi (*B. rapa* subsp. *chinensis*).

(2024). The mixture was subsequently filtered through a glass funnel to separate plant debris, and any remaining fragments were removed using forceps. The filtrate was transferred into centrifuge tubes and centrifuged at 2,000 rpm for 1 minute. The supernatant was carefully removed, leaving approximately 1.5–2 mL of sediment for further analysis. The sediment was examined under a microscope to detect nematode larvae. Microscopic observations were performed using 10× and 40× objectives, with at least three slides prepared and analyzed per vegetable sample. Experimental results were systematically recorded, and the remaining sediment was used for further analysis using Polymerase Chain Reaction (PCR) techniques.

Molecular characterization of *S. stercoralis*

Genomic DNA extraction

The DNA extraction of *S. stercoralis* from sediment samples of washing water from all ten types of vegetables was performed using the NucleoSpin® DNA Stool kit (Macherey-Nagel GmbH & Co., Duren, Germany) according to the manufacturer's instructions. The extracted DNA was then amplified by Polymerase Chain Reaction (PCR) using a specific primer pair targeting the *S. stercoralis* *cox1* gene, yielding an 836 base pair product (Laymanivong et al., 2016). A total of 30 sedimented wash-water samples from vegetables were collected for the experiment, comprising 10 samples each from Phitsanulok, Kamphaeng Phet, and Phichit provinces. The samples were derived from 10 different types of vegetables cultivated on farms in each province.

Polymerase chain reaction

All 30 samples were subjected to PCR detection analysis. Following the extraction of DNA by a NucleoSpin stool DNA kit, the nucleic acid concentrations were measured with a Nanodrop. Primers specific for the *S. stercoralis* *cox1* gene (836 bp) were used. Forward primer StrCoxAfrF (5'-GTG GTT TTG GTA ATT GAA TGG TT-3') and Reverse primer StrCoxAfrR (5'-ACC AGT TAT ACC ACC TAT AGT AA-3') were used for the amplification. (Hasegawa et al., 2009). The PCR was carried out in 20 µL reaction, which consisted of Master mix 10 µL containing Buffer MgCl₂ dNTPs (dTTP, dGTP, dCTP, and dATP) and Taq polymerase, 0.4 µL of each primer, 2 µL DNA template and 7.2 µL deionized water (18 MΩ). The PCR procedure commenced with an initial denaturation step at 94 °C for 2 minutes, followed by 30 cycles consisting of 30 seconds at 94 °C, 30 seconds at 61.5 °C, and 30 seconds at 72 °C. A final extension phase at 72 °C for 10 minutes concluded the process. Subsequently, the PCR products underwent electrophoresis on a 1.2% agarose gel set at 80 volts for 45 minutes.

Finally, the gel was stained using a nucleic acid staining solution (Red Safe™, iNtRON Biotechnology, Inc.) for analysis. The visualization of a 836 bp was considered as a positive result.

Phylogenetic analysis

The PCR products were purified utilizing the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Germany), following the manufacturer's protocol. DNA sequencing was conducted by Macrogen Inc. in Korea. Subsequently, gene sequences underwent analysis through a nucleotide BLAST search using the NCBI program Seqman II, followed by alignment using Clustal W (Thompson et al., 1994). The obtained gene sequences were compared with definitive nucleotide sequences available in The National Center for Biotechnology Information. These sequences were then utilized to construct a phylogenetic tree employing the neighbor-joining method via MEGA Version 7.0 software (Kumar et al., 2016). Additionally, the DNA sequences were submitted to the GenBank database and utilized in BLAST searches for further analysis.

RESULTS

Prevalence of Nematodes larvae infections using sedimentation technique

A total of 90 fresh vegetable samples were examined for the presence of nematode larvae contamination. The result found contamination of nematode larvae in a total of 43 samples, accounting for 47.78%. The three most contaminated types of fresh vegetables were green onions (9 samples), lettuce (8 samples), and Chinese morning glory (6 samples), corresponding to 100%, 88.89%, and 66.67%, respectively. However, this study revealed nematode larvae contamination in all types of vegetables. In Phitsanulok province, nematode larvae contamination was detected in eight types of vegetables. Green onions and lettuce were positive for nematode larvae in all three sampling periods, whereas coriander leaves and Mustard green showed no contamination. Of the 30 vegetable samples collected, 46.67% were found to be contaminated with nematode larvae, as shown in Table 1.

In Kamphaeng Phet Province, nematode larvae were detected in nine types of vegetables. Green onions and lettuce were consistently positive across all three sampling periods, whereas Chinese mustard showed no contamination. Overall, 56.67% of the 30 vegetable samples collected were contaminated with nematode larvae, as presented in Table 2.

Table 1. The detection of nematode larvae in 10 types of vegetables from Ban Dong Subdistrict, Chat Trakan District, Phitsanulok Province

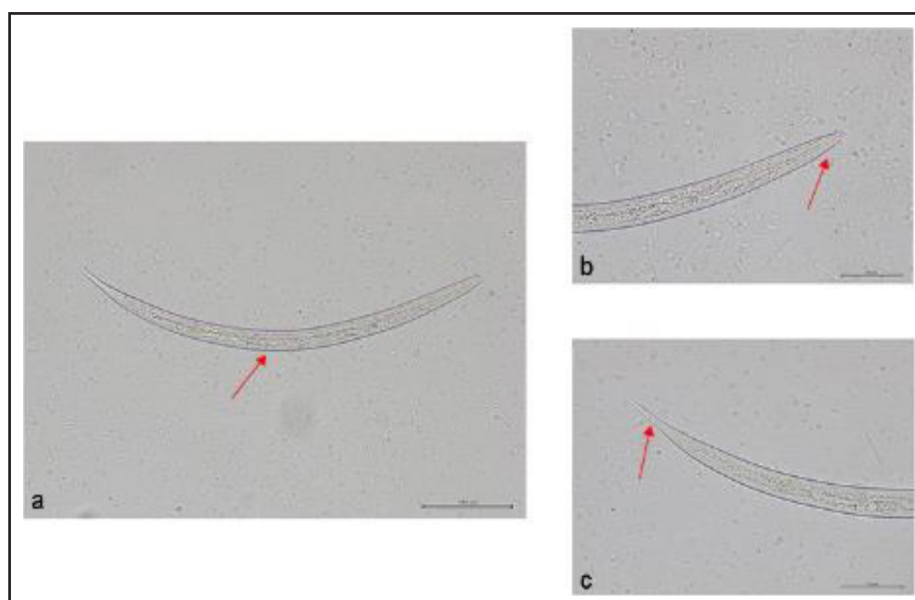
No (code)	Vegetables	Number	Nematode larvae	
			Number positive	Percentage
1. (PL1)	Chinese morning glory	3	2	66.67
2. (PL2)	lettuce	3	3	100
3. (PL3)	dill	3	1	33.33
4. (PL4)	green onion	3	3	100
5. (PL5)	celery	3	2	66.67
6. (PL6)	Chinese chives	3	1	33.3
7. (PL7)	Mustard green	3	0	0
8. (PL8)	coriander	3	0	0
9. (PL9)	gotu kola	3	1	33.3
10. (PL10)	Chinese mustard	3	1	33.33
Total		30	14	46.67

Table 2. The detection of nematode larvae in 10 types of vegetables from Wang Bua Subdistrict, Khlong Khlung District, Kamphaeng Phet Province

No (code)	Vegetables	Number	Nematode larvae	
			Number positive	Percentage
1. (KP1)	Chinese morning glory	3	2	66.66
2. (KP2)	lettuce	3	3	100
3. (KP3)	dill	3	2	66.66
4. (KP4)	green onion	3	3	100
5. (KP5)	celery	3	2	66.66
6. (KP6)	Chinese chives	3	1	100
7. (KP7)	Mustard green	3	1	33.3
8. (KP8)	coriander	3	2	100
9. (KP9)	gotu kola	3	1	100
10. (KP10)	Chinese mustard	3	0	0
Total		30	17	56.66

Table 3. The detection of nematode larvae in 10 types of vegetables from Phai Tha Pho Subdistrict, Pho Prathap Chang District, Phichit Province

No (code)	Vegetables	Number	Nematode larvae	
			Number positive	Percentage
1. (PC1)	Chinese morning glory	3	2	66.66
2. (PC2)	lettuce	3	2	66.66
3. (PC3)	dill	3	1	33.33
4. (PC4)	green onion	3	3	100
5. (PC5)	celery	3	0	0
6. (PC6)	Chinese chives	3	1	33.33
7. (PC7)	Mustard green	3	1	33.33
8. (PC8)	coriander	3	1	33.33
9. (PC9)	gotu kola	3	0	0
10. (PC10)	Chinese mustard	3	1	33.33
Total		30	12	40.00

**Figure 3.** The nematode larva observed under the microscope appeared morphologically similar to *S. stercoralis* and was detected in green onion (KP4): a. body (arrow), b. head (arrow), and c. tail (arrow).

In Phichit Province, nematode larvae were detected in eight types of vegetables. Green onions were positive in all three sampling periods, whereas celery and gotu kola leaves showed no contamination. Of the 30 vegetable samples collected, 40.00% were contaminated with nematode larvae, as shown in Table 3.

In this study, sediment was examined under light microscopy using 10× and 40× objectives. Nematode larvae were identified, and some exhibited morphological characteristics resembling *S. stercoralis*, which were detected in green onion (KP4), as shown in Figure 3.

Molecular study of *S. stercoralis* with PCR

Visualization of PCR Products

After conducting PCR with *S. stercoralis* *cox1* gene primers (StrCoxAfrF / StrCoxAfrR), the amplified products of 836 base pairs were subjected to electrophoresis. The results showed positive bands in 7 out of 30 samples (23.33%), including 3 from Phitsanulok, 2 from Phetchabun, and 2 from Phichit. In Phitsanulok Province, out of 10 samples, 3 were positive, namely PL1 (Chinese morning

glory), PL2 (lettuce), and PL4 (green onion) (Figure 4a). In Kamphaeng Phet province, 2 out of 10 samples showed bands, KP2 (lettuce) and KP4 (green onion) (Figure 4b). In Phichit Province, 2 out of 10 samples were positive, PC4 (green onion) and PC7 (Mustard green) (Figure 4c). In this study, seven samples (23.3%) tested positive for *S. stercoralis*, detected in four types of vegetables: Chinese morning glory (PL1), lettuce (PL2, KP2), green onion (PL4, KP4, PC4), and Mustard green (PC7) (Figure 4d). Subsequently, these samples were submitted for sequencing analysis.

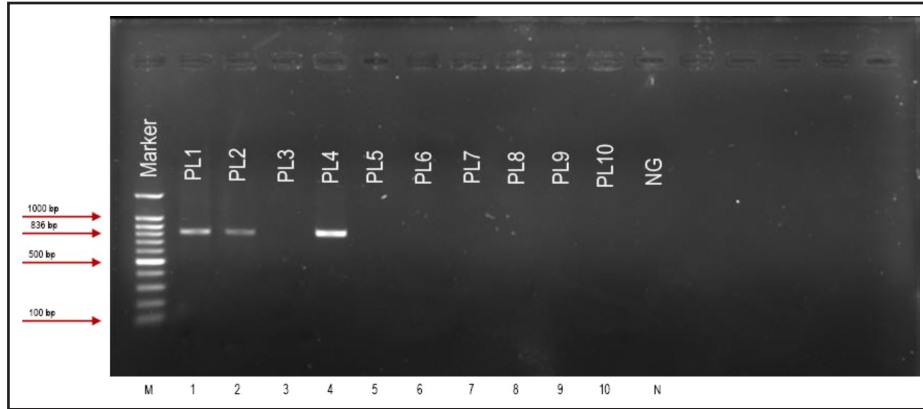


Figure 4a. PCR products in Phitsanulok samples, amplified with the primer pair StrCoxAfrF/StrCoxAfrR. Lane M, DNA Marker; lanes 1-10, vegetable samples: PL1, Chinese morning glory; PL2, lettuce; PL3, dill; PL4, green onion; PL5, celery; PL6, Chinese chives; PL7, Mustard green; PL8, coriander; PL9, gotu kola; PL10, Chinese mustard. Lane N, Negative control.

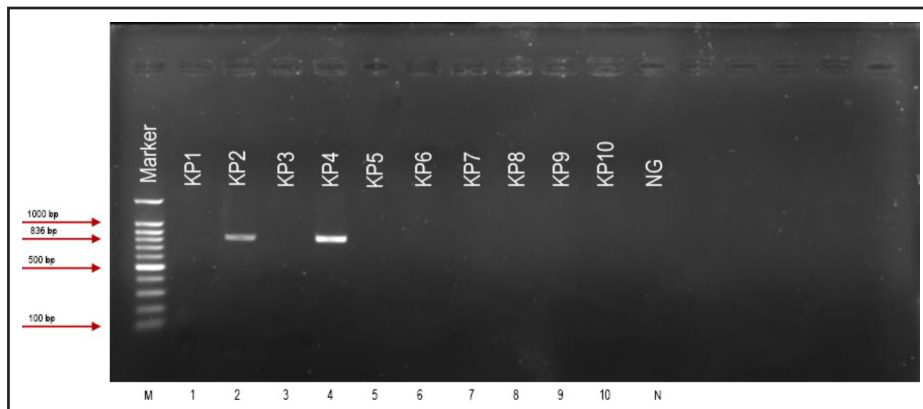


Figure 4b. PCR products in Kamphaeng Phet samples, amplified with the primer pair StrCoxAfrF/StrCoxAfrR. Lane M, DNA Marker; lanes 1-10, vegetable samples: KP1, Chinese morning glory; KP2, lettuce; KP3, dill; KP4, green onion; KP5, celery; KP6, Chinese chives; KP7, Mustard green; KP8, coriander; KP9, gotu kola; KP10, Chinese mustard. Lane N, Negative control.

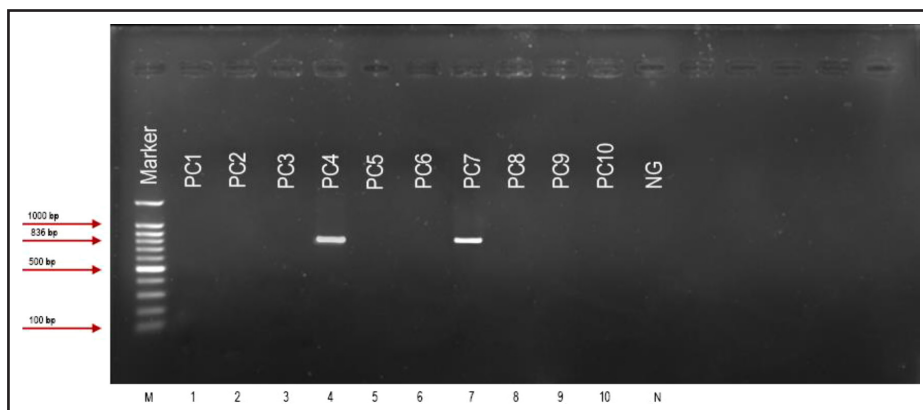


Figure 4c. PCR products in Phichit samples, amplified with the primer pair StrCoxAfrF/StrCoxAfrR. Lane M, DNA Marker; lanes 1-10, vegetable samples: PC1, Chinese morning glory; PC2, lettuce; PC3, dill; PC4, green onion; PC5, celery; PC6, Chinese chives; PC7, Mustard green; PC8, coriander; PC9, gotu kola; PC10, Chinese mustard. Lane N, Negative control.

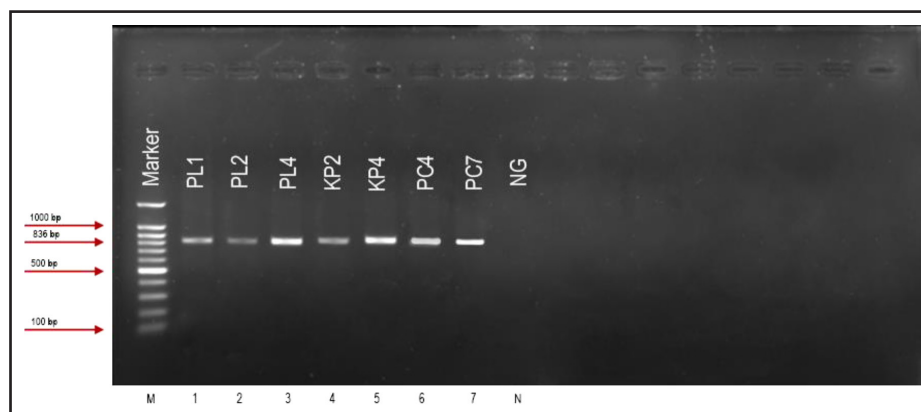


Figure 4d. PCR products in three provinces, amplified with the primer pair StrCoxAfrF/StrCoxAfrR. Lane M, DNA Marker; lanes 1-7, positive samples: PL1, Chinese morning glory; PL2, lettuce; PL4, green onion; KP2, lettuce; KP4, green onion; PC4, green onion; PC7, Mustard green. Lane N, Negative control.

Table 4. BLASTN results of *Strongyloides* spp. Larvae cox1 gene sequences in lower-northern Thailand

No.	Code	Maximum identity	BLASTN				
			Accession number	Total score	Query coverage	E value	Identity
1.	PL1	<i>Strongyloides stercoralis</i> gene, THA	KY081228.1	1454	99%	0.0	100%
2.	PL2	<i>Strongyloides stercoralis</i> gene, THA	KY081241.1	1448	99%	0.0	99.87%
3.	PL4	<i>Strongyloides stercoralis</i> gene, LAO	KU962167.1	1454	99%	0.0	100%
4.	KP2	<i>Strongyloides stercoralis</i> gene, THA	KY081241.1	1454	99%	0.0	100%
5.	KP4	<i>Strongyloides stercoralis</i> gene, THA	KY081225.1	1442	99%	0.0	99.74%
6.	PC4	<i>Strongyloides stercoralis</i> gene, THA	KY081228.1	1454	99%	0.0	100%
7.	PC7	<i>Strongyloides stercoralis</i> gene, LAO	KU962167.1	1436	99%	0.0	99.60%

DNA Sequencing and Database Searching

The sequencing results revealed that the detected organism was *S. stercoralis*, with 99-100% identity, as shown in Table 4.

Reconstruction of phylogenetic trees

The evolutionary relationships were investigated by analyzing the nucleotide sequences of the cox1 gene (836 base pairs) from all seven samples. Neighbor-Joining analysis grouped all *S. stercoralis* samples into a single clade, showing close evolutionary relationships with *S. stercoralis* sequences from Thailand (accession numbers KY081239, KY081238, KY081240, KY081241, KY081225, and KY081228), Japan (AB526304, AB526301, AB526303, and AB526302), Tanzania (AB526297 and AB526305), and Laos (KU962167). These sequences were employed to construct a phylogenetic tree using the distance-based Neighbour-Joining method, as depicted in Figure 5.

DISCUSSION

This study found nematode larvae in fresh vegetables using the sedimentation technique at a prevalence of 47.78%, which is comparable to previous reports of unidentified nematode larvae at 38.3–46.6% (Niamnuy et al., 2022; Krainara et al., 2022). A study conducted in Iran reported the presence of free-living adult nematodes (5.9%) and larvae of free-living nematodes (7.4%) (Shahnazi & Jafari, 2010). Research in Titagarh, West Bengal, India, revealed parasitic contamination in vegetables at 44.2% (Kudah

et al., 2018). In Ethiopia, contamination with *S. stercoralis* was detected in fresh vegetable samples, with carrots (63.8%) and lettuce (63.1%) showing the highest prevalence (Zeynudin et al., 2024). In Southeast Asia, a study in Hanoi, Vietnam, reported vegetable contamination at 26.0% (Uga et al., 2009). In Thailand, studies on *S. stercoralis* using the sedimentation technique revealed a prevalence ranging from 6.25% to 57% (Seawphukeaw et al., 2023; Pidsaya et al., 2025). In this study, the Sedimentation technique was found to be effective in detecting parasitic infections, and it has been reported as suitable for the detection of various types of parasites (Carneiro et al., 2018). A study conducted in Ethiopia applied the Sedimentation technique in combination with Ziehl–Neelsen staining, revealing contamination of vegetables with *S. stercoralis* (Zeynudin et al., 2024). Moreover, previous studies using light microscopy to examine morphological characteristics have reported the presence of nematode larvae, consistent with the findings of this study (Shahnazi & Jafari, 2010; Krainara et al., 2022; Niamnuy et al., 2022; Seawphukeaw et al., 2023; Gonzalez et al., 2024). In this study, nematode larvae contamination was detected in all types of vegetables examined. The three most contaminated vegetables were green onions (9 samples), lettuce (8 samples), and Chinese morning glory (6 samples), corresponding to 100%, 88.89%, and 66.67%, respectively. These findings are consistent with a study conducted in Ethiopia, which reported the prevalence of *S. stercoralis* in four types of fresh vegetables: lettuce (8%), cabbage (4%), carrot (3%), and bell pepper (3%), with a combined contamination rate of 18% (Zeynudin et al., 2024). Similarly, a study in Nakhon Si Thammarat

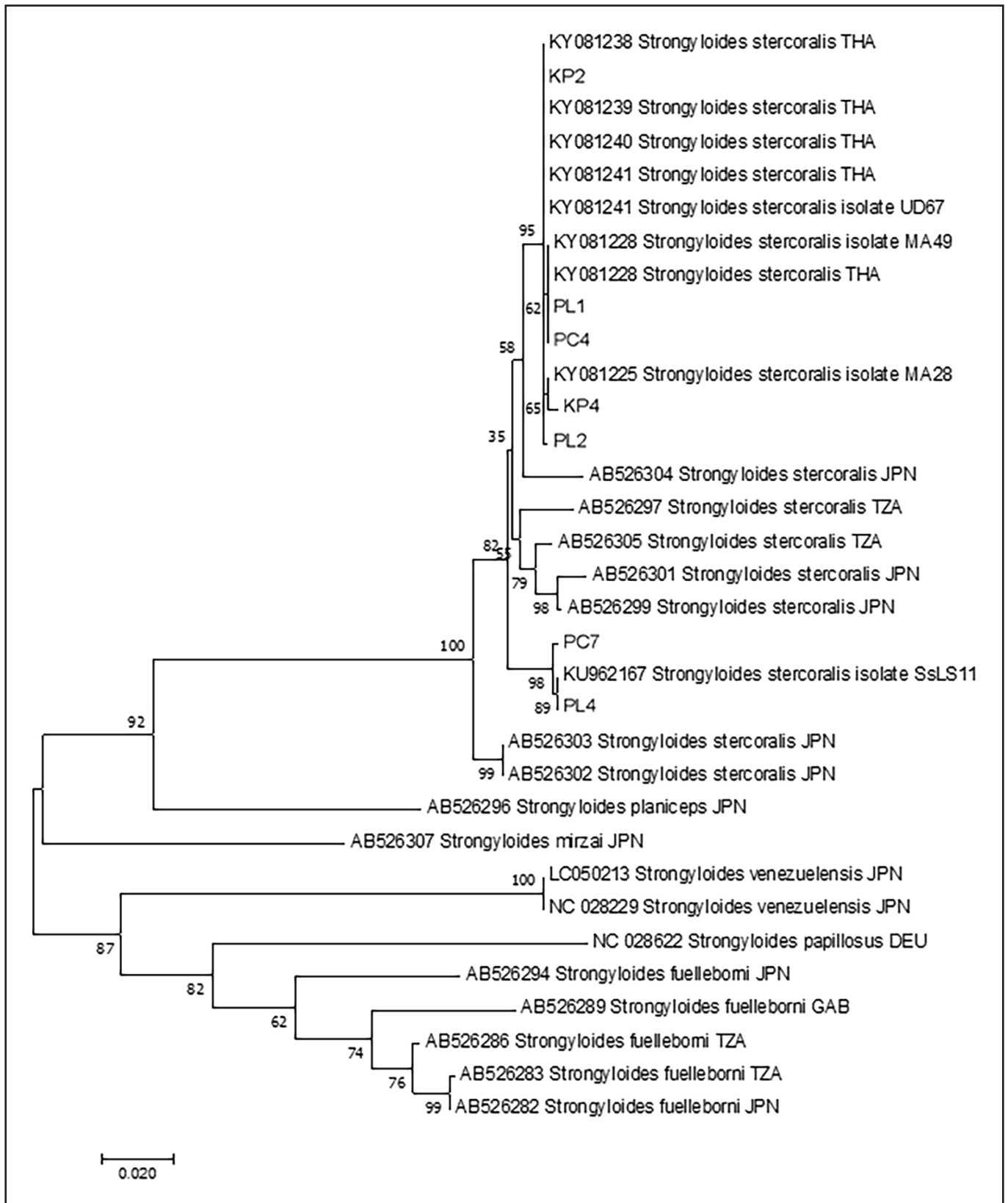


Figure 5. A Neighbor-Joining tree was constructed from the 836-base pair nucleotide sequences of the *cox1* gene of *S. stercoralis* using MEGA version 7.0, with analysis performed under the Kimura 2-parameter model and bootstrap support calculated from 1,000 replicates.

CONCLUSIONS

Province, southern Thailand, examined ten types of vegetables and found the highest contamination in celery (63.3%) and the lowest in Chinese morning glory (2.0%) (Punsawad et al., 2019). Vegetables studied in previous reports included celery, holy basil, sweet basil, mint, lettuce, parsley, coriander, spring onion, Chinese cabbage, celery, water spinach, garlic, cabbage, pennywort, kale, carrot, and cucumber (Eraky et al., 2014; Samia et al., 2017; Punsawad et al., 2019; Adogo et al., 2021; Krainara et al., 2022; Pidsaya et al., 2025). A survey conducted in Hanoi, Vietnam, in 2009 reported a high level of parasitic contamination in vegetables. Leafy vegetables showed a contamination rate of 31.0%, smooth-surfaced vegetables 30.0%, and root vegetables 17.0%. *Ascaris* spp. eggs were detected in 8.0% of samples, and unidentified parasites in 1.0% (Uga et al., 2009). In this study, contamination with nematode larvae was detected in several smooth-surfaced vegetables, including Chinese morning glory, Chinese chives, and Chinese mustard, with prevalence rates of 66.67%, 55.55%, and 22.22%, respectively. However, some vegetables with rough or textured leaf surfaces showed even higher contamination levels. The highest prevalence was observed in green onion (100%), followed by lettuce (88.89%). Dill, celery, gotu kola, and coriander showed identical contamination rates (44.44%), while Chinese cabbage had a prevalence of 22.22%. These findings suggest that certain types of vegetables may have higher contamination rates than others, possibly due to differences in leaf morphology and surface characteristics that facilitate the attachment and retention of nematode larvae. The use of human and animal feces as fertilizer was identified as a contributing factor. Similarly, a study in Qazvin, Iran, in 2010 compared parasite contamination in 436 vegetables washed with calcium hypochlorite and 218 unwashed vegetables. No parasitic contamination was detected in the washed vegetables, whereas 82 unwashed samples (37.6%) were contaminated. This indicates that consuming unwashed vegetables significantly increases the risk of parasitic infection (Shahnazi & Jafari, 2010). The molecular study of *S. stercoralis* using PCR with the primers StrCoxAfrF/StrCoxAfrR revealed an 836 bp band in 7 out of 30 samples, corresponding to 23.33%. The 836 bp band is specific to the mitochondrial DNA of *S. stercoralis*, consistent with the band size reported in studies using human stool samples (Thanchomnang et al., 2017). In Malaysia, *S. stercoralis* has been investigated using real-time PCR to confirm microscopic observations of the parasite (Zeehaida et al., 2011). Another study extracted *S. stercoralis* DNA from stool samples using PCR primers specific for the 18S ribosomal RNA gene, namely MSP4f and StrongR, which amplify a 568 bp fragment (Laymanivong et al., 2016). In the present study, DNA was extracted from the washing water of vegetables containing multiple nematode larvae, which is a more practical approach compared to extracting DNA from a single filariform larva (Thanchomnang et al., 2017). The confirmation of *S. stercoralis* infection in vegetable wash sediments using PCR revealed specific bands in four types of vegetables: Chinese morning glory (PL1), lettuce (PL2, KP2), green onion (PL4, KP4, PC4), and Mustard green (PC7). For detection, the Cox1 primers (836 bp) were used, and sequencing of the PCR products followed by BLAST analysis in NCBI confirmed all samples as *S. stercoralis*, showing 99–100% identity. Phylogenetic analysis using the Neighbor-Joining method grouped all *S. stercoralis* samples into a single clade, demonstrating close evolutionary relationships with reference sequences. Sequencing further indicated that these strains are closely related to those reported in the Republic of Lao, Tanzania, and Japan, suggesting genetic diversity within the species. The phylogenetic findings highlight genetic affinities with neighboring countries, emphasizing the importance of comprehensive public health strategies for surveillance, treatment, and prevention of parasite transmission through vegetables. Moreover, this study provides insight into the molecular characteristics of *S. stercoralis*, revealing close genetic relationships among strains from various Southeast Asian countries, which may reflect regional transmission patterns and evolutionary relationships.

In this study, nematode larvae were detected in fresh vegetables using the sedimentation technique, with an overall prevalence of 47.78% based on morphological examination under a light microscope. Among these, larvae resembling *S. stercoralis* were observed in four types of vegetables: Chinese morning glory, lettuce, green onion, and Mustard green. However, nematode contamination was found in all vegetable types examined. The three most heavily contaminated vegetables were green onions (9 samples, 100%), lettuce (8 samples, 88.89%), and Chinese morning glory (6 samples, 66.67%). The molecular study of *S. stercoralis* was conducted using PCR with the primers StrCoxAfrF/StrCoxAfrR, which amplified a product of 836 bp in 7 out of 30 samples, corresponding to 23.33%. Sequencing results confirmed that the detected organism was *S. stercoralis*, showing 99–100% identity. Furthermore, a phylogenetic analysis of the seven vegetable-derived samples collected from three provinces was performed using the Neighbor-Joining method, which also confirmed the samples as *S. stercoralis*, revealing close genetic relationships among strains from various Southeast Asian countries.

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Conflict of interests

We declare that we have no conflict of interest.

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