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Assessment of the global paradigms of genetic variability in *Strongyloides stercoralis* infrapopulations determined by mitochondrial DNA sequences

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ABSTRACT

Microevolutionary data of *Strongyloides stercoralis* infrapopulations are regarded as a requirement for determining the global genetic structure and transmission paradigms of this neglected tropical nematode among the neighboring countries of the world. English databases were searched from 2010 to 2019, analyzing a total of 10 publications. The cytochrome c oxidase subunit 1 sequences of *S. stercoralis* isolated from Asian and African continents were subjected to calculate the diversity indices and genetic differentiation. A parsimonious haplotype network indicated a star-like trait a total of 106 (*Homo sapiens*) and 48 haplotypes (Canid) being grouped into four distinct geographical haplogroups. A significant genetic diversity was identified in human-derived *S. stercoralis* (Haplotype diversity: 0.78) and those with dog (Hd: 0.86) origins. Cladistic phylogenetic tree indicated the Japanese, Thailandish, and Myanmarese clades have a sister relationship with the Laotian clade. The statistically significant *F*st values indicated that human *S. stercoralis* populations of Japanese-Thailandish, Japanese-Myanmarese, and Japanese-Laotian origins were genetically differentiated (*F*st: 0.48430 to 0.54903). We conclude that a high gene migration of human strongyloidiasis is being unequivocally shared between the Laotion-Myanmarese and Laotion-Thailandish population pairs. The current findings enhance our knowledge to assess the transmission dynamics and the evolutionary patterns of *S. stercoralis* in various geographical regions of the globe; also it will serve as a basis for public health policy to control human strongyloidiasis particularly in immunocompromised individuals. Besides, the infected canids and other environmental reservoirs for zoonotic transmission of *S. stercoralis* to humans should be de-wormed along with their owners.

1. Introduction

Based on the World Health Organization (WHO) report, *Strongyloides stercoralis* has been categorized as one of the 17 most neglected nematodes, mostly distributed in tropical (2%–25%) and subtropical (0.4%–4.0%) areas, where sewage disposal is inadequate or absent [1–3]. The global prevalence of *S. stercoralis* has estimated about 350 million cases over 70 different countries including the tropical regions of South-East Asia, Africa, and Latin America [1–4]. *S. stercoralis* is considered an opportunistic nematode in immunocompromised patients which can be related to a high mortality rate [2]. The life cycle of *S. stercoralis* is perpetuated between parasitic and free-living generations via four distinct transmission routes including homogonic cycle (Non-sexual direct route), heterogonic cycle (Sexual indirect route), internal autoinfection, and external autoinfection. The parasitic female adults produce parthenogenetic offspring that finally develop into infective third-stage filariform larvae [5,6].

To date, molecular phylogeny approaches determined by mitogenome markers of metazoan have yielded the most well-documented and reliable data about epizootiology, intra-and inter-species diversity, evolutionary biology, and population structure of parasites [7–11]. Although considerable efforts have been locally made to reveal the genetic data and population origin of *S. stercoralis*, nonetheless according to our current data, little has been gained from the comparative phylo-geographical study on gene flow of *S. stercoralis* haplotypes among the endemic areas of the world [12–17]. The cytochrome c oxidase subunit 1 (*Cox1*) is a well-known evolutionary mitogenome marker for accurate perception of taxonomic status and phylogeny of helminthic infections, which are represented by their low repetitive sequences in length genome, being haploid, their higher rate of

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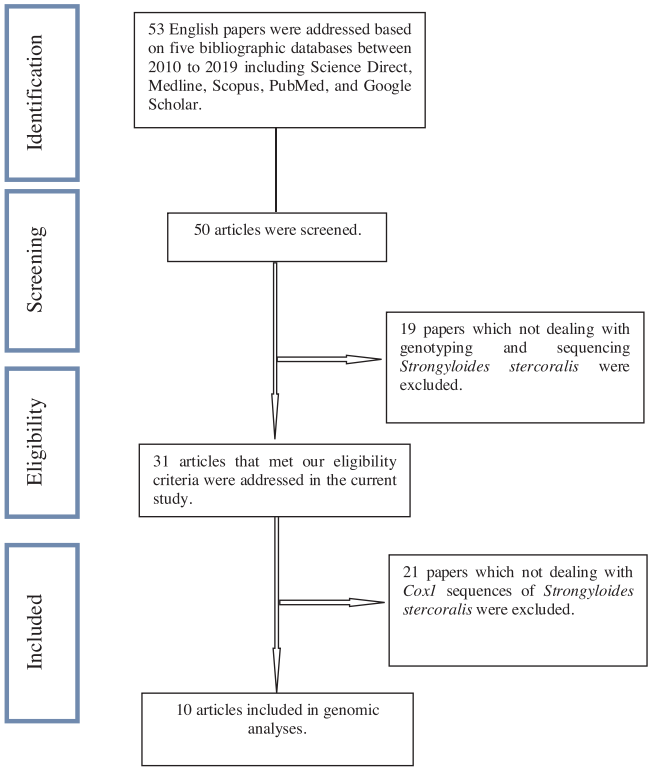

Fig. 1. Flowchart describing the study design process.

mutation, and relatively high evolutionary rate [7,8,18,19].

This systematic review characterized the available nucleotide sequences of *Cox1* data on the genetic heterogeneity and population pattern of *S. stercoralis* derived from *Homo sapiens* and canid hosts to investigate how this nematode is continentally circulating across the Africa, South-East, and Middle-East Asia.

2. Materials and methods

2.1. Search strategy

English databases including Science Direct, Scopus, Google Scholar, MEDLINE, and PubMed Central were explored for the articles published between 2010 and 2019, leading to the identification of a total of 53 publications (Fig. 1). The following MeSH keywords were considered in the initial search strategy: "*Strongyloides stercoralis*", "canine strongyloidiasis", "human strongyloidiasis", "Genetic diversity/Genetic variation", "Gene migration", "Population genetic structure", "Phylogeography", "Intraspecific variation", "Genetic polymorphism", "Mitochondrial *Cox1* DNA sequences".

2.2. Data extraction and genomic analysis

Cox1 nucleotide sequences of *S. stercoralis* extracted from published and unpublished papers were directly retrieved from GenBank database. A total of 614 *S. stercoralis* nucleotide sequences of the *Cox1* (*Homo sapiens*: 488 and dog: 126) were downloaded in FASTA format [7,14,16,17,20–23]. Nucleotide sequences were grouped according to human and canine strongyloidiasis originating from East Asia (Japan and China), Africa (Tanzania, Central African Republic, and Uganda) South-East Asia (Thailand, Myanmar, Laos, and Cambodia), and Middle-East Asia (Iran) (Table 1).

Ambiguous sites among the target nucleotide sequences were aligned using Clustal W using Sequencher™v.4.1.4 Software. The analysis of molecular variance (AMOVA) of exported sequences was

Table 1
Global geographical *Cox1* sequences of *Strongyloides stercoralis* used in this review based on phylogeographical and type of host.

Country	Parasite	Host	Accession number	Reference
China	*Strongyloides stercoralis*	*Homo sapiens*	MK040537-MK040539	[29]
Cambodia	*Strongyloides stercoralis*	*Homo sapiens*	KX226367- KX226373	Unpublished
Iran	*Strongyloides stercoralis*	*Homo sapiens*	LC476975-LC476977, MH921561-MH921564, MG995852 and KP663661	Unpublished [20]
Japan	*Strongyloides stercoralis*	*Homo sapiens*	LC179148-LC179154, LC179026- LC179028, LC179535- LC179538, and LC179542, LC179385- LC179392, LC179393, LC179365- LC179384, LC179360 -LC179364, LC179231- LC179235, AB526298-AB526301	[7,16]
Laos	*Strongyloides stercoralis*	*Homo sapiens*	KU962160-KU962180, KU962140-KU962159, KU962139,	[14]
Myanmar (Burma)	*Strongyloides stercoralis*	*Homo sapiens*	LC179126-LC179147, LC179069-LC179088, LC179110-LC179125, LC179087-LC179092, LC179066- LC179068, LC179093-LC179109, LC179046-LC179065, LC179029-LC179045, LC179504-LC179511, LC179512-LC179526, LC179470- LC179485, LC179499-LC179502, LC179459-LC179469, LC179222-LC179225, LC179166-LC179180, LC179217-LC179221, LC179200- LC179216, LC179180-LC179183, LC179184-LC179199	[16]
Tanzania	*Strongyloides stercoralis*	*Homo sapiens*	AB526297	[7]
Uganda	*Strongyloides stercoralis*	*Homo sapiens*	LC179527-LC179534	[16]
Thailand	*Strongyloides stercoralis*	*Homo sapiens*	LC179345-LC179358, LC179254-LC179344, KY081224-KY081239, LC179308, LC179278	[16,17]
Cambodia	*Strongyloides stercoralis*	Dog	KX226374-KX226384	[12]
Myanmar	*Strongyloides stercoralis*	Dog	LC179488-LC179498, LC179022, LC179486-LC179487, LC179441-LC179455, LC179421-LC179440, LC179401-LC179420, LC179394-LC179400, LC179241-LC179243, LC179236-LC179240	[16]
Japan	*Strongyloides stercoralis*	Dog	LC179022-LC179025,LC179155-LC179159, LC179456-LC179458, LC179244,LC179245-LC179253, LC179251, LC179230,LC179160-LC179165, LC179226-LC179229	[12,16]

Table 2
Diversity and neutrality indices of *Strongyloides stercoralis* isolated from canids based on nucleotide sequences of *Cox1* gene. N: number of isolates; Hn: number of haplotypes; Hd: haplotype diversity; Nd: nucleotide diversity.

Country Continent		Diversity indices					Neutrality indices	
		N	Hn	Hd ± SD	Number of segregating sites	Nd (π)	Tajima's D*	Fu's Fs statistic**
Asia	Cambodia	12	11	0.985 ± 00.040	61	0.04183	−0.33647	−0.732
	Japan	32	3	0.421 ± 0.030	24	0.01318	2.00543	16.299
	Myanmar	85	34	0.946 ± 0.011	84	0.01994	−0.70113	−2.414
Total		126	48 (38%)	Average, Hd; 0.868				

NC: Not calculable.
* Not significant, 0.10 > P > 0.05.
** Not significant, P > 0.10.

Table 3
Diversity and neutrality indices of *Strongyloides stercoralis* isolated from *Homo sapiens* based on nucleotide sequences of *Cox1* gene. N: number of isolates; Hn: number of haplotypes; Hd: haplotype diversity; Nd: nucleotide diversity.

Country Continent		Diversity indices					Neutrality indices	
		N	Hn	Hd ± SD	Number of segregating sites	Nd (π)	Tajima's D*	Fu's Fs statistic**
Asia	China	3	2	1.000 ± 0.272	2	0.00242	NC	NC
	Japan	58	7	0.461 ± 0.061	26	0.00353	−1.77820	1.383
	Cambodia	7	7	1.000 ± 0.076	18	0.076	0.93086	−1.847
	Laos	40	28	0.953 ± 0.022	78	0.01541	−1.22132	−7.432
	Iran	9	6	0.889 ± 0.091	18	0.02427	−0.91042	0.163
	Myanmar	234	29	0.876 ± 0.016	45	0.01418	0.93644	1.425
	Thailand	124	23	0.900 ± 0.014	56	0.01516	0.11964	2.164
Africa	Central African Republic	4	3	0.833 ± 0.222	13	0.01654	−0.60528	1.960
	Tanzania	1	NC	NC	NC	NC	NC	NC
	Uganda	8	1	0.000	0	0.000	0.000	0.000
	Total	488	106 (21.7%)	Average, Hd; 0.78				

NC: Not calculable.
* Not significant, 0.10 > P > 0.05.
** Not significant, P > 0.10.

Table 4
Pairwise *F*st values (bottom left) and estimated number of migrants (Nm) per *Strongyloides stercoralis* isolated from *Homo sapiens* (top right) of worldwide population.

Country	Populations Japan	Laos	Myanmar	Thailand
Japan	–	0.21	0.27	0.21
Laos	0.54903	–	17.24	39.52
Myanmar	0.48430	0.01430	–	5.56
Thailand	0.54496	0.00629	0.04302	–

calculated by DnaSP software (version 5.10) to estimate the neutrality indices (Tajima's *D* and Fu's Fs) and genetic diversity indices (Haplotype diversity (Hd); nucleotide diversity, (π)) [24]. The pairwise fixation index (F-statistics: *F*st) was calculated to evaluate the grade of genetic differentiation within the various geographical populations of *S. stercoralis*. To determine the genealogical associations of intra-specific diversity of *S. stercoralis*, a haplotype network was drawn inferred by the Median-Joining algorithm (PopART software) [25]. To demonstrate a cladistic relationship between the human and canid sequences of *S. stercoralis*, a maximum likelihood tree was built in MEGA7 software [26]. *Strongyloides fuelleborni* was addressed as an outgroup branch (Accession number., AB526294). Bootstrap values of more than 60% supported by the topology of the constructed tree. The pairwise sequence distance including intra-divergence and percent identity among the various geographical sequences of the *Cox1* was built using the DNA Star Meg Align program.

3. Results

3.1. Study selection process and genomic analysis

A total of 53 publications relate to *S. stercoralis* were initially identified. Following the exclusion of three duplicates generated from the databases, the titles of 50 papers were screened and 21 articles which did not deal with *Cox1* sequences of *S. stercoralis* were excluded (Fig. 1). Finally, the full texts of the remaining 10 publications were selected and *Cox 1* (n = 614) nucleotide sequences were subjected to genomic analysis. The 711 bp consensus sequences of *S. stercoralis* isolates were selected. Sequence analysis of *Cox1* gene clarified that 154 unique haplotypes were identified from human (n: 106; 21.7%) and dog (n: 48; 38%) within the Asian and African clades (Tables 2 and 3). No insertion/deletion (*I*ndel) mutations were identified at consensus sequence; while transversion and transition substitutions including synonymous and non-synonymous mutations were occurred among the aligned sequences.

3.2. Diversity indices, genetic differentiation, and pairwise sequences distances

Diversity and neutrality indices for *S. stercoralis* obtained from human and canid (*Canis lupus* familiaris) hosts are shown in Tables 2 and 3. Analyses of the *Cox1* nucleotide sequences showed significant genetic diversity in human-derived *S. stercoralis* isolates (Haplotype diversity: 0.78) and dog (Hd: 0.86) origins, which principally occurred in Asian isolates, whereas nucleotide diversity was low in entire populations (π: 0.00242 to 0.04183) (Tables 2 and 3). However, a genetic uniformity of *S. stercoralis* (Hd: 0.00) was detected in the human isolates (n = 8) originating from Uganda. Tajima's D (−0.70113 to

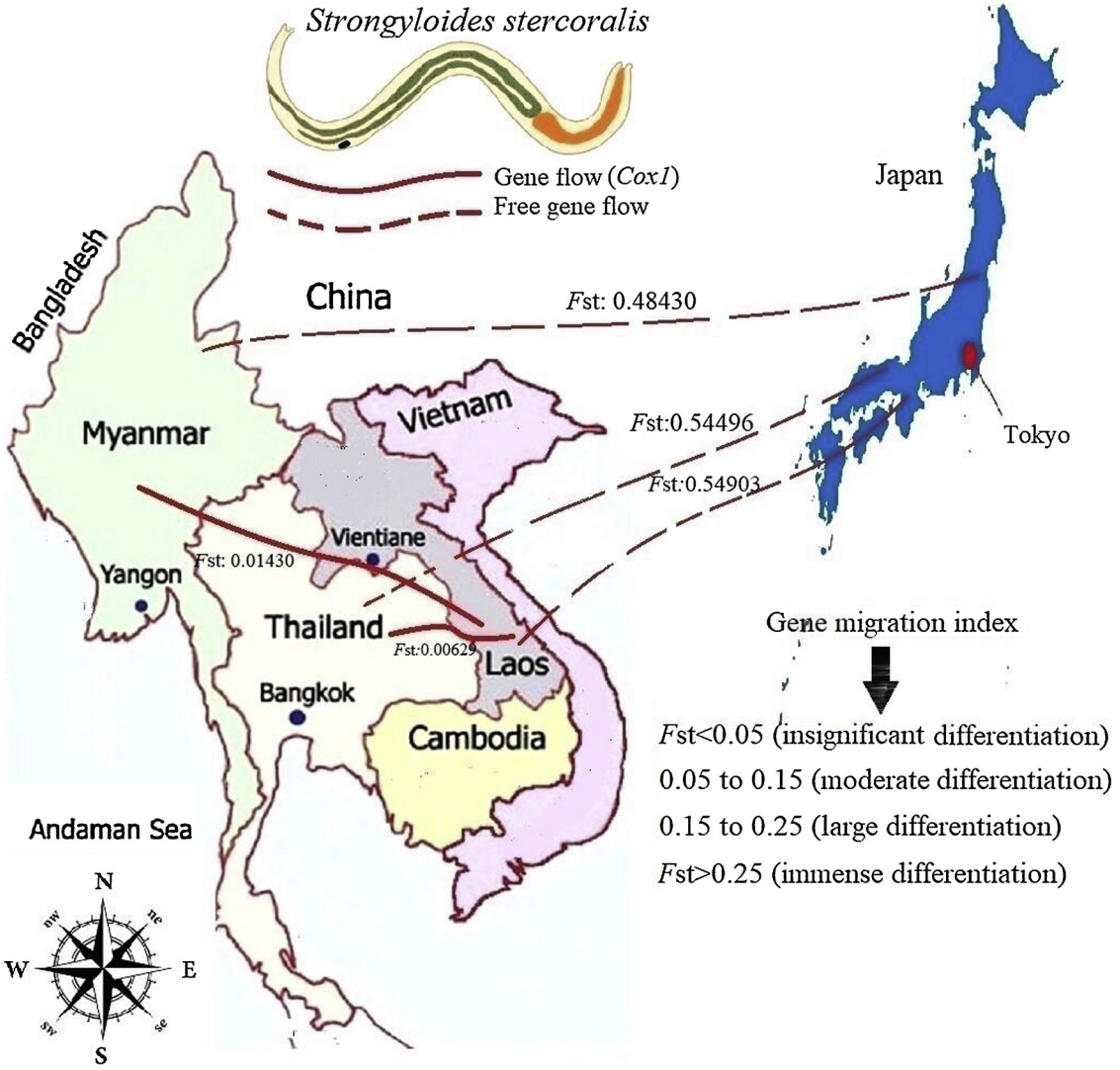

Fig. 2. Map of Far East Asia and South East Asia presenting the gene (flow) migration of *Strongyloides stercoralis Cox1* haplotypes among endemic regions.

Table 5
Pairwise *F*st values (bottom left) and estimated number of migrants (Nm) per *Strongyloides stercoralis* isolated from dog (top right) of worldwide population.

Country	Populations		
	Japan	Laos	Myanmar
Japan	–	0.44	0.10
Laos	0.36229	–	0.79
Myanmar	0.70445	0.23970	–

−1.22132) and Fu's Fs (−7.432 to −0.732) neutrality indices for the *Cox1* haplotypes demonstrated negative values in *S. stercoralis* derived from Laos, Myanmar, and Cambodia, suggesting a considerable divergence from neutrality (Tables 2 and 3). The globally pairwise sequence distance matrix amongst *S. stercoralis* sequences obtained from *H. sapiens* showed an intra-species divergence of 0%–4% and percent identity of 96%–100%, while a significant intra-species divergence of *S. stercoralis* was estimated (0%–8.1%) in canine strongyloidiasis.

The statistically significant *F*st (a gene flow index) values indicated that human *S. stercoralis* populations originated from Japanese-Thailandish, Japanese-Myanmarese, and Japanese-Laotians origins were genetically differentiated (*F*st: 0.48430 to 0.54903), although no genetic differentiation was observed between Laotion-Myanmarese (*F*st: 0.01430) and Laotion-Thailandish (*F*st: 0.00629) population pairs (Table 4). The statistically significant *F*st values of canine strongyloidiasis showed that the populations of Japanese-Cambodian, Japanese-Myanmarese, and Cambodian-Myanmarese origins were genetically differentiated (*F*st: 0.23970 to 0.70445) (Fig. 2). The highest genetic differentiation of human strongyloidiasis was observed between Laotion-Japanese (*F*st: 0.54903) and Thailandish-Japanese (*F*st: 0.54496) population pairs, while highest genetic differentiation of canine strongyloidiasis was observed between Japanese-Myanmarese (*F*st: 0.70445) (Table 5).

3.3. Phylogenetic tree and haplotype network

Distance-based Maximum Likelihood cladistic tree of *H. sapiens S. stercoralis* sequences indicated the *Cox1* haplotypes were assigned into Thailandish (Clade I), Japanese (Clade II), Myanmarese (Clade III), and Laotion (Clade IV) clades (Fig. 3). Cladistic phylogenetic tree indicated the Japanese, Thailandish, and Myanmarese clades have a sister relationship with the Laotians clade (Fig. 3). A parsimonious haplotype network showed a star-like aspect with a total of 106 (*H. sapiens*; 21.7%) and 48 haplotypes (Dog; 38%) being classified into four distinct geographical haplogroups (I; Thailand, II: Iran and Central Africa, III; Laos and IV; Cambodia). The haplotype network analysis indicated the spread of the haplotypes Ss1 (AB526297) and Ss2 (KY081234) from Tanzania and Thailand to Laos haplogroup, likewise, the haplotype Ss7

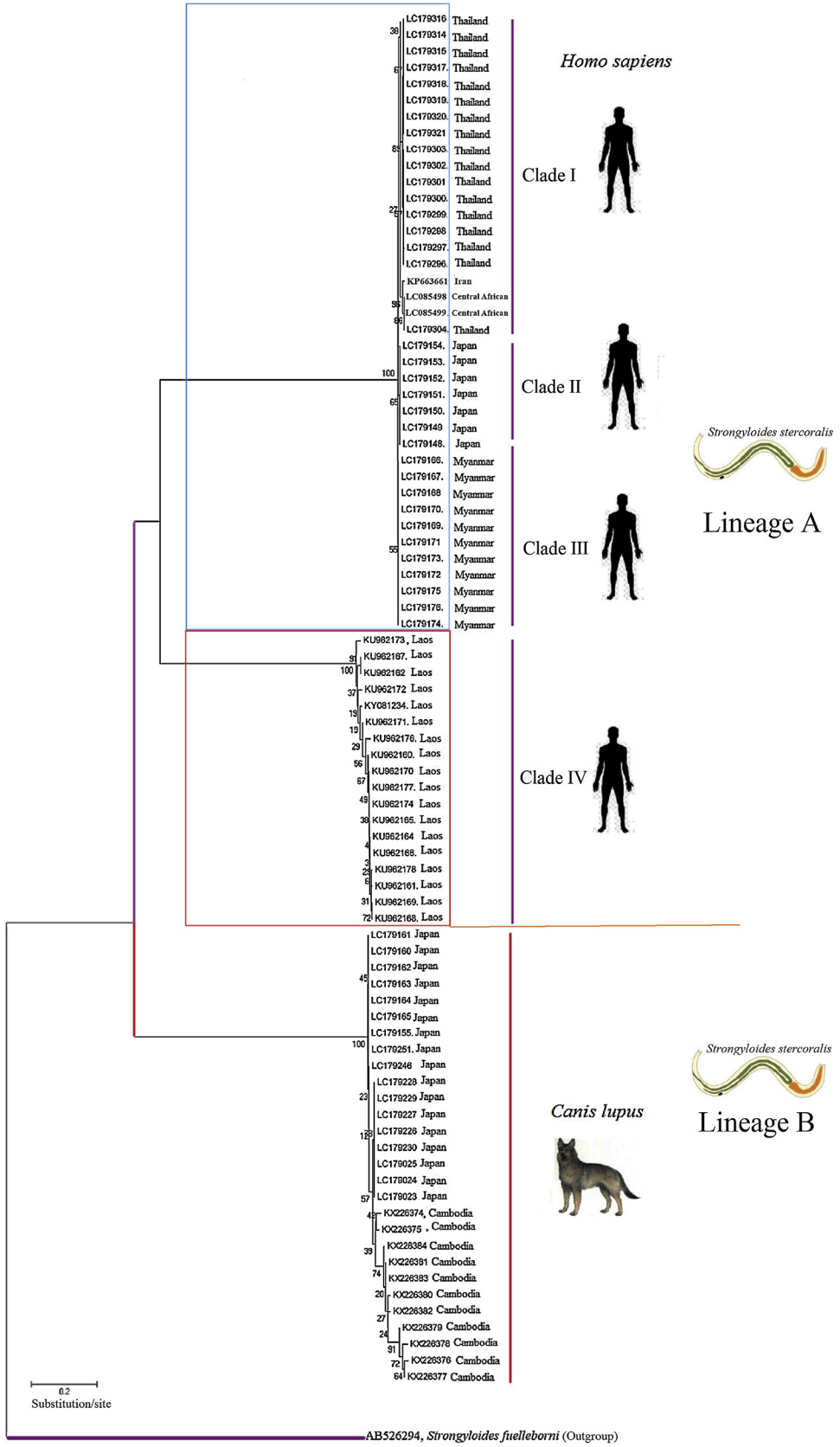

Fig. 3. A phylogenetic tree of *Strongyloides stercoralis* based on *Cox1* sequences in globally circulating isolates. Two genetically distinct lineages of A and B separated based on host specificity (*Homo sapiens* and canid). Values on the tree nodes are bootstrap proportions (%). Only bootstrap values higher than 60% are indicated on each branch.

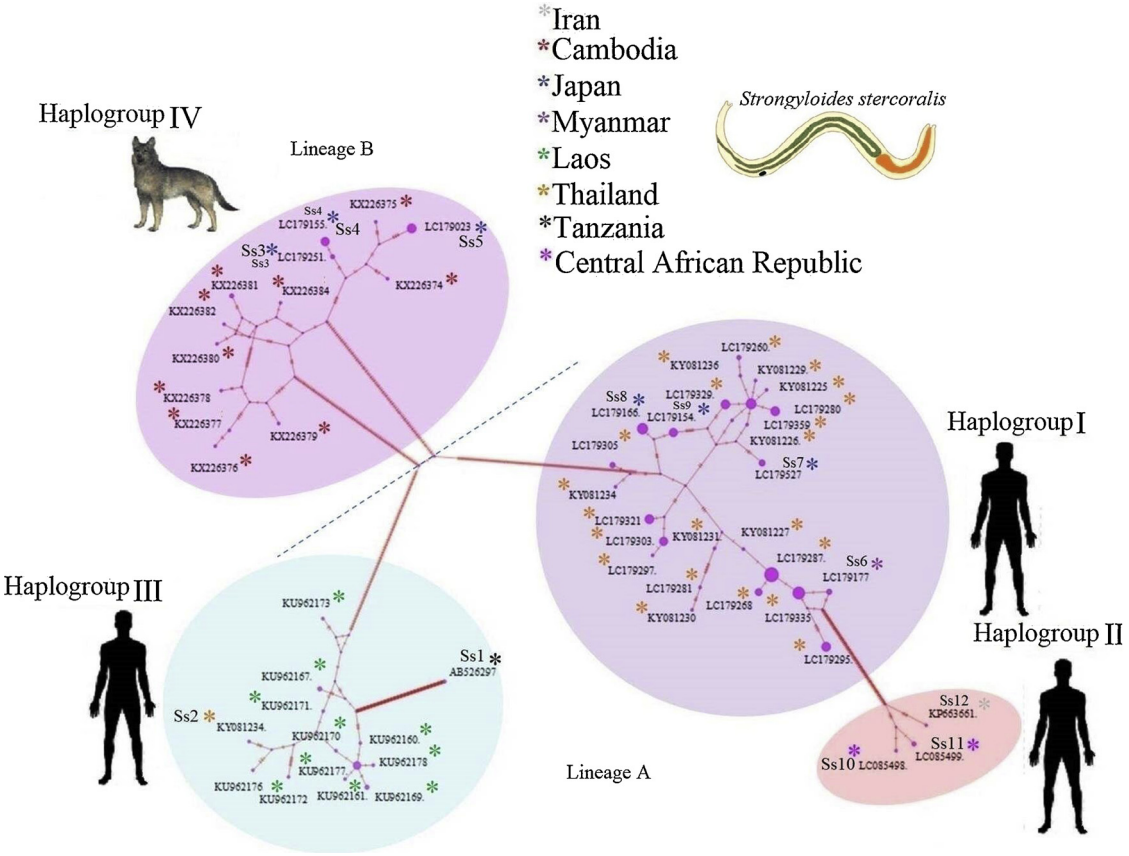

Fig. 4. A parsimonious haplotype network of *Strongyloides stercoralis* based on *Cox1* sequences in globally circulating isolates. Various geographical haplotypes were marked by asterisk (*).

(LC179527) extended from the Central African Republic to Thailand haplogroup (Fig. 4; marked by an asterisk). The obtained haplotypes from canine strongyloidiasis (Haplogroup IV) were segregated far from the human strongyloidiasis haplotypes (Haplogroups I, II, and III).

4. Discussion

Investigating the genetic heterogeneity data and intra-species diversity within human and canine strongyloidiasis spur a fundamentally biological interest to identify the transmission dynamics (i.e. insect control and dog de-worming) of this neglected parasite to design novel drug targets and potential prevention programs [8,13]. The generated population genetic datasets of *S. stercoralis* allowed us to propose an evolutionary hypothesis on how the divergent *Cox1* haplotypes of *S. stercoralis* originating from *H. sapiens* and canid hosts respond to selective pressure. The mitochondrial genomic *Cox1* is a valuable DNA barcode for revealing intraspecific mutants and provides new insights for studying geographical populations of *S. stercoralis* [7,8,13].

It has also been documented that high genetic heterogeneity can frequently occur following gene migration in the structural biology of microbial pathogens since high gene flow increases the effective population size in various geographical areas, where the diversity indices can potentially dominate [9,11]. Contrary, a low degree of genetic diversity was estimated in both human (Hd: 0.461) and canine (Hd: 0.421) strongyloidiasis originating from Japan. This level of diversity possible arose from current selective sweeps, in which some functional alleles drift great swathes of the genome to near-fixation owing to widespread linkage disequilibrium.

Our haplotype network data indicate that *S. stercoralis* haplogroup IV originated from Cambodian dogs (Lineage B; *Canis lupus)* have not zoonotically been adapted to infect *H. sapiens*, and this might be justified by the ancestral phenotype of this nematode. Therefore, to complete this theoretical transmission puzzle, further investigation is essential to the development of next-generation sequencing and multilocus microsatellite typing of *S. stercoralis* infrapopulations from humans and canids from various geographical regions based on non-adaptive genomes. Recently, Nagayasu et al., (2017) have explained that a distinct lineage of *S. stercoralis* (type B) originated from Myanmarese dogs has not been adapted to infect human, while type A parasite is able to infect both humans and dogs in Southeast Asia, Japan, and Africa [16]. Also, they have possibly surmised that human *S. stercoralis* had been formerly developed as a canid nematode, and later was spread into humans [16].

Additionally, the *F*st values showed that human strongyloidiasis originated from Japanese-Thailandish, Japanese-Myanmarese, and Japanese-Laotians origins were genetically differentiated (*F*st: 0.48430 to 0.54903; $P < 0.05$). This genetic diversity differences may be attributed to climatic diversity (tropical/subtropical), moisture content, and the temperature of the region's soils. A similar study demonstrated *S. stercoralis* isolated from Japanese patients (subtropical) has comparatively a greater genetic differentiation from the nematode isolated from Thailand patients (tropical) [15]. Genome-wide analyses has previously been employed to study the population genetics of *S. stercoralis* isolates, in which a relatively genetic diversity of this parasitic nematode from Japanese samples have been probably justified by the independent development of two haplotypes of diploid genomes during auto-infection cycle (asexual reproduction), indicating the study on genetic heterogeneity traits are necessary to surmise infection history and geographical prevalence [27].

Furthermore, the insignificant *F*st values showed that there is no genetic difference in human strongyloidiasis between Laotion-Myanmarese and Laotion-Thailandish population pairs. We conclude

that a high gene migration (syn., gene flow; genetic drift) of human strongyloidiasis *Cox1* haplotypes by the infectious individuals' traveling to mentioned areas is unequivocally circulating among the Laotion-Myanmarese and Laotion-Thailandish origins. The highest gene flow of *Cox1* haplotypes was observed (*F*st: 0.00629 to 0.01430) in human strongyloidiasis between Laotion-Myanmarese and Laotion-Thailandish population pairs. On the one hand, the statistically significant *F*st values (0.48430 to 0.54903) indicated that there is no gene migration of human *S. stercoralis Cox1* haplotypes between Japanese-Thailandish, Japanese-Myanmarese, and Japanese-Laotians population pairs. This would suggest that the countries of Laos, Myanmar, and Thailand share a common border with each other which it stands to reason that their *Strongyloides* would be closely related to each other compared to isolates from Japan. Furthermore, following the quick globalization in diverse countries, host mobility, the ecological changes, further examination of the environmental reservoirs, trading of infected canids, and occurrence of a bottleneck effect can be probably considered as predisposing agents of *S. stercoralis* dispersal across the mentioned areas [15,28].

Cole et al., (2018) have suggested that since environments have a tendency to change, the genetic range of parasitic nematodes can reduce following habitat fragmentation populations because of the smaller effective population size of hosts [19].

The attendance of considerable negative neutrality indices (Tajima's D; −0.70113 to −1.22132; Fu's Fs −7.432 to −0.732) in support of all canine (Cambodia and Myanmar) and human (Laos) strongyloidiasis represents an excess of low frequency of *Cox1* haplotypes compared to the expectations under neutral developments such as population size equilibrium, model of neutral mutation, and population expansion following the bottleneck effect [9–11,28]. The expansion of the haplotypes Ss1 (Accession no., AB526297) and Ss2 (Accession no., KY081234) from Tanzania and Thailand to Laos's haplogroup, along with extension of haplotype Ss7 (Central African Republic; Accession no., LC179527) in Thai populations supports the likelihood of these haplotypes' appearance in the neighboring countries and their transmission into the mentioned populations. Secondly, haplotypes Ss1, Ss2, and Ss7 have emerged in each region, being derived from their common haplotypes.

In this review, because of the inadequate numbers of the registered *Cyt b* and ATPase1 mitochondrial sequences in the GenBank database, we could not comprehensively calculate the concatenated markers to assess the various features of the genetic drift in *S. stercoralis* populations. Besides, because of this limitation, we could not authentically conclude resultant genetic data of *S. stercoralis* particularly at risk people such as immunosuppressed patients, hyperinfection syndrome, AIDS, HIV, HTLV-1, transplant recipients, and corticosteroid users.

The current findings enhance our knowledge to assess the transmission dynamics, dispersion of plausible drug-resistant mutants, and the evolutionary paradigms of *S. stercoralis* in different geographical regions of the globe; also it will become the basis of public health policy to control human strongyloidiasis particularly in immunocompromised and HIV positive individuals. Besides, the infected dogs and Caniformia (dog-like animal) as potential reservoirs for zoonotic transmission of *S. stercoralis* to humans should be de-wormed along with their owners.

Declaration of Competing Interest

The authors declare that there is no conflict of interests.

Acknowledgments

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