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Review Article

Leishmaniasis in Thailand: A Review of Causative Agents and Situations

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Abstract. Before 1999, leishmaniasis was considered an imported disease in Thailand. Since then, autochthonous leishmaniasis was reported in both immmunocompetent and immmunocompromised patients especially in human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS). A new species was identified and named as *Leishmania siamensis* consisting of two lineages, that is, lineages TR and PG. Analysis of isoenzymes has clarified the more commonly detected *L. siamensis* lineage PG as *Leishmania martiniquensis* (MON-229), a species originally reported from the Martinique Island, whereas the *L. siamensis* lineage TR has been identified as the true novel species, *L. siamensis* (MON-324). Both cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) have been found among Thai patients. Disseminated CL and VL could be presented in some reported patients who had HIV/AIDS coinfection. So far, only sporadic cases have been reported; thus, the true prevalence of leishmaniasis should be determined in Thailand among the high-risk populations such as people with HIV/AIDS. A recent survey among animals identified *L. martiniquensis* DNA in black rats (*Rattus rattus*) suggesting a potential animal reservoir. In addition, *L. martiniquensis* DNA was identified in *Sergentomyia gemmea* and *Sergentomyia barraudi*, the predominant sandfly species in the affected areas. However, further studies are needed to prove that these sandflies could serve as the vector of leishmaniasis in Thailand.

INTRODUCTION

Leishmania is an intracellular protozoa, a member of the Family Trypanosomatidae, Order Kinetoplastida. The transmission occurs by the bite of the phlebotomine female sandfly. Two subgenera, *Leishmania* (*Leishmania*) and *Leishmania* (*Viannia*), the members of the subgenus *Leishmania* develop in the midgut region, whereas those of subgenus *Viannia* develop in the hindgut region of sandflies.[1] *Leishmania* causes three major clinical forms of infection, that is, cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL). VL or kala-azar, the most severe form, is caused by parasite multiplication in the reticuloendothelial system, mainly the liver, spleen, lymph node, and bone marrow. VL causes a broad range of symptoms from asymptomatic to a fatal outcome.[2,3] Symptoms depend on host immunity and species of the parasite.[3–5] Concomitant human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) not only increases the risk of active VL but also results in poor responses to treatment.[4,5]

More than 20 species of *Leishmania* have been determined as human pathogens, endemic in the Middle East, Central and North America, Indian subcontinent, and Mediterranean basins. Currently, 0.9–1.3 million new cases are annually reported with 20,000–30,000 deaths caused by VL.[6,7] Primary causative agents of VL are *Leishmania donovani* complex species including *L. donovani* and *Leishmania infantum* in the Old World and *Leishmania chagasi* (synonym *L. infantum*) in the New World. *Leishmania tropica* and *Leishmania amazonensis* primarily cause CL, but could be viscerotropic in some cases.[8,9] In Thailand, leishmaniasis mainly constituted imported cases before 1999. This review emphasizes the emerging autochthonous leishmaniasis in Thailand. To date, two newly identified species, *Leishmania martiniquensis* and *Leishmania siamensis* have caused autochthonous leishmaniasis among Thai patients. The data presented in this review were obtained from published articles of leishmaniasis in Thailand from 1999 to 2016 in PubMed.

SPECIES IDENTIFICATION OF *LEISHMANIA* (*LEISHMANIA*) *MARTINIQUENSIS* AND *LEISHMANIA* (*LEISHMANIA*) *SIAMENSIS*

With regard to species identification, two axenic cultured promastigote isolates from two VL patients[10] were sent for isoenzyme analysis at the French Reference Center on Leishmaniasis, Montpellier, France. These isolates were reported as two lineages of *L. siamensis*: lineage PG and TR.[10] The results of 15 isoenzyme analyses revealed two distinct species. The first lineage, *L. siamensis* lineage PG (MHOM/TH/2011/PG), was identical to the zymodeme MON-229 of *L. martiniquensis* (MHOM/MQ/92/MAR1). *Leishmania martiniquensis* is a species originally reported from Martinique Island[11] in an HIV-infected patient with diffused nodular CL in 1995.[12] Other molecular typings of three target genes of *L. martiniquensis* (MHOM/MQ/92/MAR1) and the GenBank accession numbers are as follows: RNA polymerase (AF326982), DNA polymerase (AF326983),[13] and 18S-rRNA (AF303938).[11]

Although the *L. siamensis* lineage TR was confirmed as *L. siamensis* (MHOM/TH/2010/TR, zymodeme MON-324), a novel species was first reported in Thailand (Figure 1).[14] Other molecular typings of the three protein coding DNA sequences of *L. siamensis* (MON-324) are as follows: locus 03.0980, elongation initiation factor 2 a-subunit (JQ586200); locus 04.0580, spermidine synthase 1 (JQ586201); and locus 31.2610 and RNA polymerase II largest subunit (JQ586202).[14] Details of isoenzyme analysis using 15 enzymes and molecular markers of the two species as well as other biological characteristics of *L. martiniquensis* and *L. siamensis* are summarized in Table 1.[15–31]

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TABLE 1
A summary of the biological characteristics of *Leishmania martiniquensis* and *Leishmania siamensis*

	L. martiniquensis		*L. siamensis*	
	Characteristic	Reference	Characteristic	Reference
Geographical distribution	Martinique island/French West Indies (human cases)	11	Thailand (a human case)	14
	Thailand (human cases)	15, 18–20, 22, 26–29		
	Germany, Switzerland (horses, cow)	16, 17		
	United States (a horse)	21		
	Myanmar (human cases)	20, 23		
Clinical type in HIV seronegative	VL	15, 22		
	CL	20, 23		
Clinical type in HIV seropositive	DCL and VL	19, 20, 26, 28	DCL and VL	14
	VL	18, 27, 30		
	CL	20		
Clinical type in animals	CL*	16, 17, 21	No data	
Size of amastigote (light microscopy)	Diameter: 3.99 ± 0.48 μm	11	No data	
Axenic in vitro cultivation	SDM79 medium supplemented with 15% fetal calf serum, 7 μg/mL hemin, and 2.5 μg/mL 6-biopterin or NNN medium or Schneider's medium supplemented with 20% fetal calf serum	11	NNN medium or Schneider's media supplemented with 20% fetal calf serum	Unpublished data
Size of promastigote in culture	Body length: 9.44 ± 3.02 μm; body width: 2.20 ± 0.63 μm; flagellum length: 11.59 ± 3.63 μm	11	Body length: 7.29 + 1.53 μm; body width: 1.22 + 0.29 μm; flagellum length: 9.80 + 2.52 μm	Unpublished data
Enzyme profiles*	MDH^{150}, ME^{45}, ICD^{95}, PGD^{87}, $G6PD^{78}$, $GLUD^{300}$, DIA^{30}, $NP1^{00}$, $NP2^{85}$, $GOT1^{170}$, $GOT2^{00}$, $GM1^{04}$, FH^{65}, MPI, $^{13}GPI^{52}$	11	MDH^{112}, ME^{70}, ICD^{100}, PGD^{140}, $G6PD^{85}$, $GLUD^{260}$, DIA^{20}, NP_1^{00}, NP_2^{120}, GOT_1^{122}, GOT_2^{122}, $PGM^{109/96}$, FH^{60}, MPI^{137}, $GPI^{88/76}$	Unpublished data
WHO Code	MHOM/MQ/92/MAR1	11	MHOM/TH/2010/TR	Unpublished data
	MHOM/TH/2011/PG (enzyme profiles similar to those of MHOM/MQ/92/MAR1)	Unpublished data		
Zymodeme	MON-229 [MHOM/MQ/92/MAR1]	11	MON-324	Unpublished data
	MON-229 [MHOM/TH/2011/PG]	Unpublished data		
Molecular typing	RNA polymerase (AF32698)	13	Elongation initiation factor 2 a-subunit (JQ586200)	14
	DNA polymerase (AF326983)	13	Spermidine synthase 1 (JQ586201)	
	18S rRNA (AF303938)	11	RNA polymerase II largest subunit (JQ586202)	
Phylogenetic analysis	Closely related to *Leishmania enrietti* complex	27	Closely related to *Leishmania enrietti* complex	14
Experimental vertebrate host	Infective to BALB/c mice and amastigotes were detected in the popliteal and mesenteric lymph nodes, liver, spleen, and brain	31	No data	
Potential animal reservoir	Black rats (*Rattus rattus*) (detection by PCR in blood, liver, and spleen)	25	No data	
Potential sandfly vector	*Sergentomyia gemmea*, *Sergentomyia barraudi* (detection by PCR in sandfly)	24, 25	No data	

BALB/c mice = an albino, laboratory-bred strain of the house; CL = cutaneous leishmaniasis; DCL = diffused cutaneous leishmaniasis; HIV = human immunodeficiency virus; NNN = Novy-MacNeal-Nicolle medium; PCR = polymerase chain reaction; VL = visceral leishmaniasis; WHO = World Health Organization.
*Isoenzyme analysis was performed at French Reference Center on Leishmaniasis, UMR5290, Montpellier, France.

As shown in Table 2, all *L. siamensis* published papers from 2008 to 2016[14–29] were reviewed and the species of *Leishmania* were confirmed using nucleotide sequences of the SSU-rRNA, ITS1 region of SSU-rRNA, and *hsp*70 genes from GenBank database and were compared with the nucleotide sequences of each gene using axenic cultures of the reference species, *L. siamensis* [MON 324] (SSU-rRNA/JQ280883, ITS1/JQ001751, and *hsp*70/KC202880) and *L. martiniquensis* [MON-229] (SSU-rRNA/JN885899, ITS1/JX195637, and *hsp*70/KC202882). The results confirmed that 12 *Leishmania* infections in Thai and Myanmar patients were *L. martiniquensis* as well as

TABLE 2
Reported and clarified *Leishmania* species reported in humans, animals, and sandflies

Reference	Host	Area	GenBank accession numbers			Reported species	Clarified species§
			SSU-rRNA*	ITS1†	*hsp*70‡		
15	Human	Phang Nga, Thailand	JN885899¶	EF200012	N/A	A suspected novel species	*L. martiniquensis*
16	Horse	Germany, Switzerland	N/A	GQ281278 GQ281279 GQ281280 GQ281281	N/A	Closely related to *L. siamensis*	*L. martiniquensis*
17	Cow	Switzerland	N/A	GQ281282	N/A	A suspected novel species	*L. martiniquensis*
18	Human	Chanthaburi, Thailand	GQ226033	GQ226034	N/A	A novel species	*L. martiniquensis*
19, 20	Human	Songkhla, Thailand	N/A	JQ001751	N/A	A novel species	*L. martiniquensis*
19, 20	Human	Trang, Thailand	N/A	JQ001752‖	N/A	A novel species	*L. martiniquensis*
21	Horse	Florida	N/A	JQ617283	N/A	*L. siamensis*	*L. martiniquensis*
14	Human	Trang, Thailand	JQ280883	JX195640	KC202880	*L. siamensis*	*L. siamensis*
20	Human	Myanmar	GQ226033	N/A	N/A	*L. siamensis*	*L. martiniquensis*
20	Human	Chiang Rai, Thailand	N/A	N/A	N/A	*L. siamensis*	*L. martiniquensis*
20	Human	Myanmar	N/A	N/A	N/A	*L. siamensis*	*L. martiniquensis*
22	Human	Stun, Thailand	JN087497	JX195637	KC202882	*L. siamensis*	*L. martiniquensis*
20, 23	Human	Myanmar	N/A	JQ001751	N/A	*L. siamensis*	*L. martiniquensis*
24	Sandfly (*Sergentomyia gemmea*)	Trang, Thailand	N/A	N/A	JX852708	*L. siamensis*	*L. martiniquensis*
25	Sandfly (*Sergentomyia barraudi*)	Songkhla, Thailand	N/A	JQ866907	N/A	*L. siamensis*	*L. martiniquensis*
25	Black rats (*Rattus rattus*)	Trang, Thailand	N/A	JQ866906	N/A	*L. siamensis*	*L. martiniquensis*
26	Human	Southern Thailand	N/A	JQ001751	N/A	*L. siamensis*	*L. martiniquensis*
27	Human	Lamphun, Thailand	N/A	JX898938	N/A	*L. martiniquensis*	*L. martiniquensis*
28	Human	Chiang Mai, Thailand	N/A	KJ 210834 KJ210835	KP244367	*L. martiniquensis*	*L. martiniquensis*
28	Human	Lamphun, Thailand	N/A	KJ 210836 KJ210837	KP244368	*L. martiniquensis*	*L. martiniquensis*
29	Human	Thailand	N/A	KU050863	N/A	*L. martiniquensis*	*L. martiniquensis*

L. martiniquensis = *Leishmania martiniquensis*; *L. siamensis* = *Leishmania siamensis*; N/A = not available.
*Small subunit ribosomal RNA gene.
†Internal transcribed spacer 1 of SSU-rRNA gene.
‡Heat shock protein 70 gene.
§Clarified species by sequence analysis using GenBank database of the three genes; *L. siamensis* (SSU-rRNA/JQ280883, ITS1/JQ001751, *hsp*70/KC202880) and *L. martiniquensis* (SSU-rRNA/JN885899, ITS1/JX195637, and *hsp*70/KC202882).
¶Unpublished accession number from Sukmee and others (2008), first referred in Bualert and others (2012).
‖The sequences of accession number JQ001752 and KF227887 to KF227892 were obtained from the specimens collected from the same source but different site of specimen collection. All sequences showed 100% identical.

those reported in horses and cow.[16,17,21] Only one published paper by Bualert and others in 2012 identified *L. siamensis* infection.[14]

PHYLOGENETIC ANALYSIS

Phylogenetic trees based on three genetic loci, that is, SSU-rRNA, ITS1, and *hsp*70, including sequencing data of *L. siamensis* and *L. martiniquensis* available in GenBank have been reconstructed (Figure 2). The SSU-rRNA tree was constructed using three *L. martiniquensis* isolates, one *L. siamensis* isolate, and 10 reference sequences of different *Leishmania* species. The phylogenetic analyses showed that both *L. martiniquensis* and *L. siamensis* isolates were grouped together in monophyletic clade implying that their evolutionary processes seemed not to be related to other *Leishmania* species. The molecular and isoenzymatic techniques of *L. martiniquensis* clearly show that this species belongs to the subgenus *Leishmania*. Hence, the grouping of *L. siamensis* together with *L. martiniquensis* in the same monophyletic branch primarily suggests that *L. siamensis* is closely related to members of the same subgenus. However, the SSU-rRNA sequences are unable to discriminate between these two *Leishmania* species due to the 100% identical sequences (data not shown) indicating that the variation of these gene fragments used to construct phylogenetic tree limits the classification of these parasites at the species level (Figure 2A). The characteristic of this phylogenetic tree was supported by the fact that two pairs of *Leishmania major* and *Leishmania amazonensis*; *Leishmania guyanensis* and *Leishmania panamensis* sequences were also 100% identical.

The reconstruction of a phylogenetic tree based on ITS1 region including the *L. martiniquensis* reference sequence together with 13 other *Leishmania* reference sequences has revised the relationship among the *L. siamensis* cases previously analyzed. The phylogenetic tree showed that the *L. martiniquensis* reference sequence was grouped with the same taxa that have been identified as *L. siamensis* suggesting that most isolates would belong to *L. martiniquensis*. Figure 2 includes the *Leishmania* sequences obtained from sandflies and black rats in the study of animal reservoirs and potential vectors of *L. siamensis* by Chusri and others (JQ866907, JQ866906)[25] and sequences belong to *L. siamensis* lineage PG previously isolated from Thai patients (GQ226034, GQ293226, JQ001751, and JQ001752),[10,18,19] horses (JQ617283, GQ281278, GQ281279, GQ281280, and GQ281281),[16,21] and cows (GQ281282).[17] However, the *L. siamensis* lineage TR,

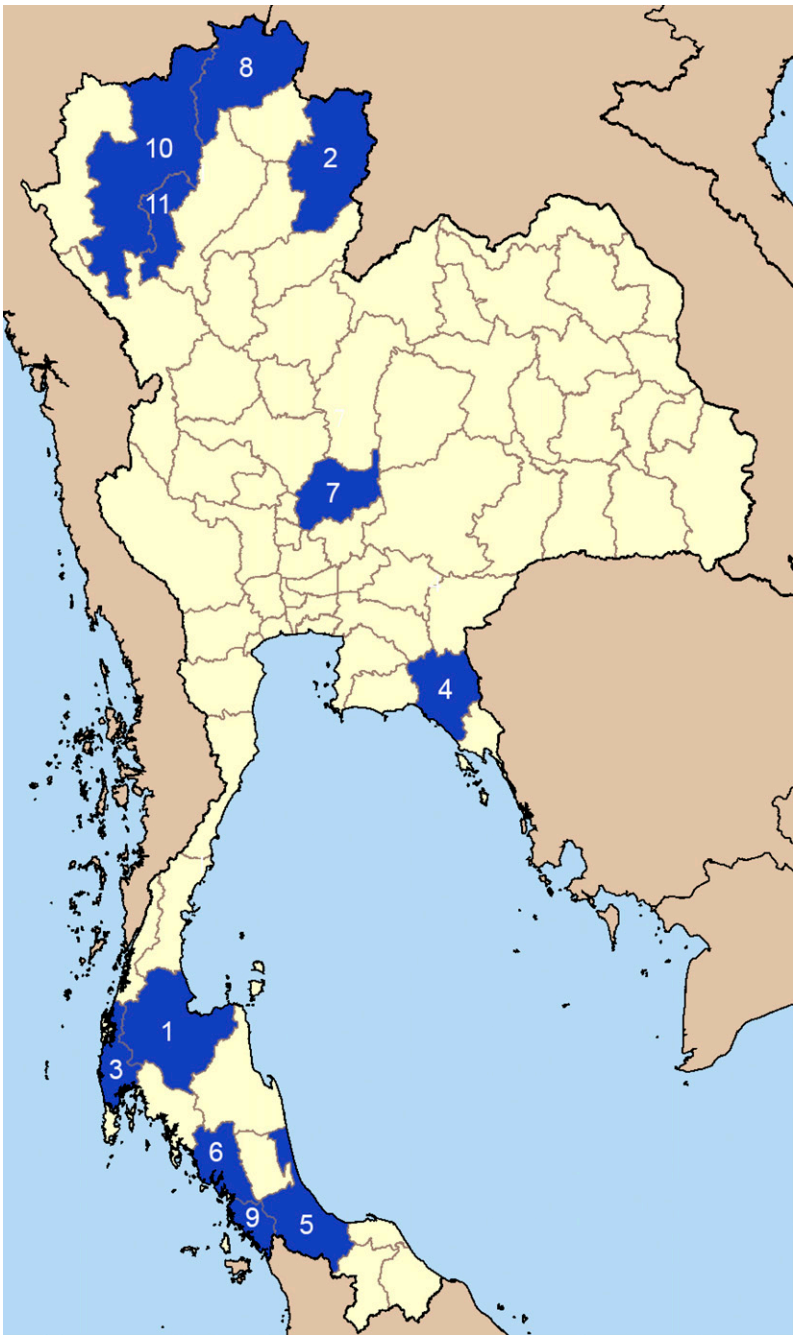

FIGURE 1. Locations of the reported autochthonous leishmaniasis cases in Thailand. 1. Surat Thani,[32] 2. Nan,[33] 3. Phang-nga,[15] 4. Chanthaburi,[18] 5. Songkhla,[19,29] 6. Trang,[14,19] 7. Lopburi,[34] 8.Chiang Rai,[20] 9. Stun,[22] 10. Lamphun,[27,28] 11. Chiang Mai.[28]

now termed *L. siamensis*, still forms a separate branch from *L. martiniquensis* indicating the close relationship between the two species (Figure 2B).

A few studies have focused on the *hsp*70 gene compared with the ITS1 for species identification. The information obtained from this gene has been considered one of the most useful data to provide precise relationship of genus *Leishmania*. Although no reference sequence of *L. martiniquensis* is available in the database, the *hsp*70 sequences of *Leishmania* isolates PCM2, CU1, and PCM4 that have been identified the species by ITS1 region could be logically considered as representative sequences of *L. siamensis* and *L. martiniquensis*, respectively. These would implicitly make the case reported by Leelayoova and others (KC202881)[10] and the sequence obtained from the potential vector[24] to be *L. martiniquensis* (Figure 2C).

SITUATION OF LEISHMANIASIS IN THAILAND

Before 1999, both CL and VL were considered imported diseases. A retrospective review showed 40 CL and 6 VL cases were reported from 1960 to 1997.[35] Of 46 cases,

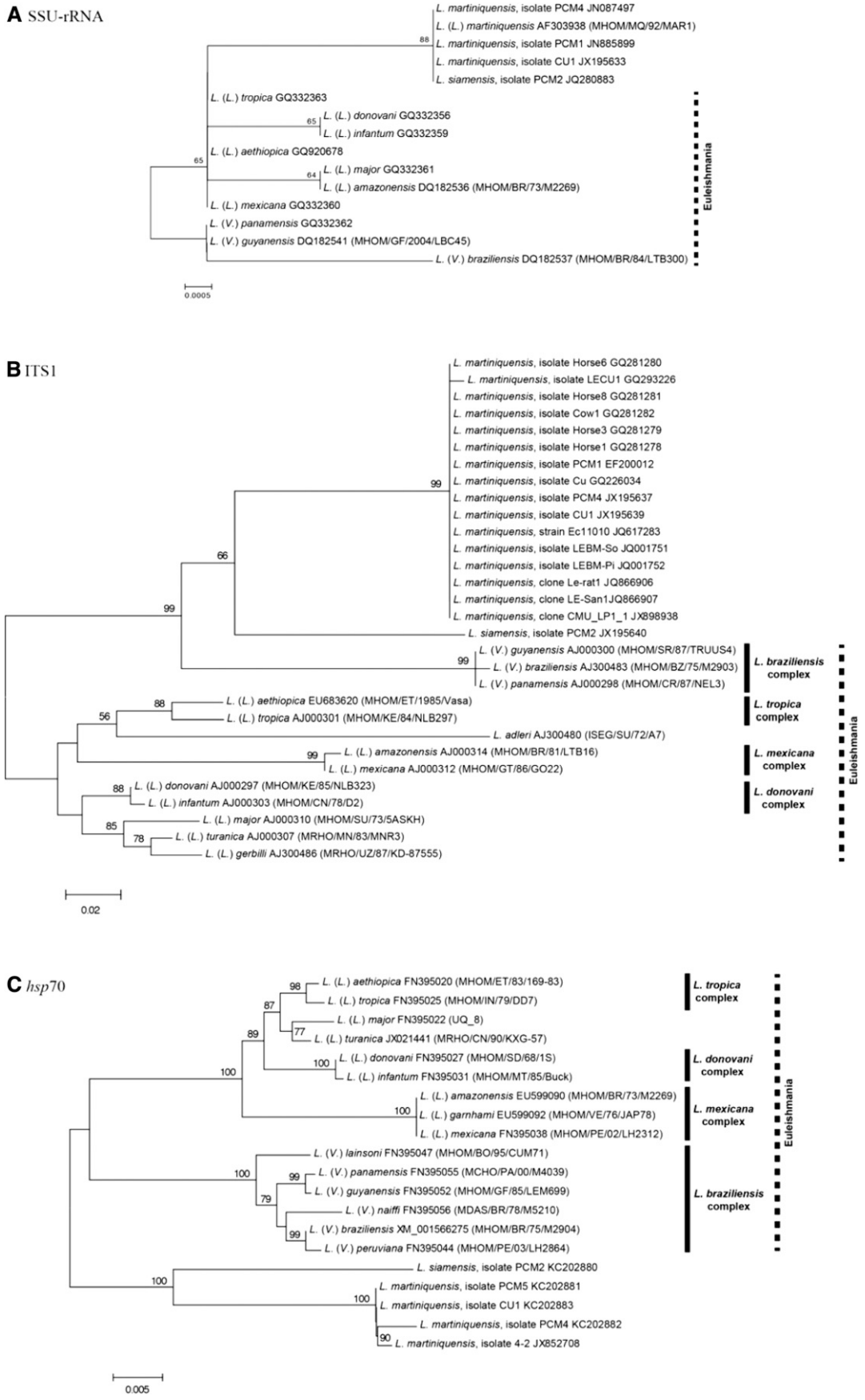

FIGURE 2. The unrooted phylogenetic tree inferred from DNA sequences of three markers: (**A**) SSU-rRNA, (**B**) ITS1, and (**C**) *hsp70* using neighbor-joining method.

44 comprised Thai workers who returned from endemic areas, that is, Saudi Arabia, Iraq, and Libya. A public health concern of *Leishmania* infection in Thailand started when the first two autochthonous VL were reported in 1999 and 2007.[32,33] However, species identification was not performed. In 2008, a suspected new species of *Leishmania* causing autochthonous VL in an immunocompetent Thai patient was reported in Phang Nga Province, southern Thailand.[15] The map of Thailand shows sporadic cases of CL and VL reported in six southern, one central, one eastern, and four northern provinces. Table 3 shows the characteristics of published leishmaniasis cases in Thailand from 1999 to 2016. Approximately 90% of cases comprised adults with an age range from 30 to 81 years, although the youngest patient was a 3-year-old girl. More than 50% of them were male, lived in the south, and about half of these cases comprised people with HIV/AIDS. All documented VL cases had chronic infection and developed severe symptoms. However, some VL patients who had coinfection with HIV/AIDS could develop disseminated dermal leishmaniasis. In the coinfected patients, most cases had CD4+ counts less than 200 cells/μL. Of these cases, one autochthonous CL was reported in a 5-year-old girl. In 2008, one VL case caused by *L. infantum* was reported in a Thai male patient living in Bangkok, central Thailand,[36] where source of infection, biological vector, and reservoir host could not be identified due to his traveling history. Thus, this case was not considered as autochthonous and was excluded from the map. In addition, a CL was reported in a 3-year-old girl; however, the causative agent was not identified.[34]

Interestingly, one case involved a Burmese male patient living in Yangon, Myanmar, who came to Thailand to seek medical care. He was an immunocompetent host who developed multiple erythematous and nodules on his face, trunk, and extremities after receiving steroid therapy, and afterward received a diagnosis of diffused cutaneous leishmaniasis (DCL) caused by *L. martiniquensis*.[20,23] His daughter, who lived in the same house, also had asymptomatic *L. martiniquensis* infection.[20] Until now, *L. martiniquensis* infection has been reported in different geographical areas, that is, Martinique Island/French West Indies, Thailand, and Myanmar (Table 1).

With regard to *L. siamensis*, only one DCL and VL was recorded in a person with AIDS[14]; however, our preliminary survey revealed a number of asymptomatic VL caused by *L. siamensis* in immunocompetent and immunocompromised individuals (J. Manomat and others, unpublished data). Public health personnel should be aware of *L. siamensis* transmission and be ready for a substantial increase in the numbers of symptomatic cases in the future.

CLINICAL SPECTRUM OF *L. MARTINIQUENSIS* AND *L. SIAMENSIS* INFECTIONS

CL in immunocompetent patients, caused by *L. martiniquensis*, shows lesions present only at the site of inoculation on the skin and is called localized CL (LCL) (S. Natesuwan and others, unpublished data). However, the majority of lesions on skin in HIV/AIDS cases could be single, multiple nodular, or generalized papular forms. The lesion could be disseminated as multiple nonulcerative nodules, papular with ulcerative lesions or chronic generalized fibrotic lesions to other parts of the body besides a primary lesion on the face, ears (along the helix and antihelix of the pinna), the dorsum of hands, knuckles of fingers, elbows, extensor surface of the forearm, or on the trunk and lower extremities,[19,20,23,28] called DCL. For *L. siamensis* infection, only one DCL was reported in a person with AIDS. Thick and hard skin nodules covering the body especially on the face, trunk, and extremities were observed. Histological examination revealed diffused irregular hard subcutaneous nodules varying in size.[14]

Clinical characteristics of VL caused by *L. martiniquensis* are similar to typical VL reported in *L. donovani* and *L. infantum* for which main clinical symptoms include prolonged fever, anemia, hepatosplenomegaly, and cachexia. Due to thrombocytopenia, some patients could have

TABLE 3
Documented cases of leishmaniasis reported from Thailand during 1999–2016

Case no.	Age (years)	Sex	Province	Clinical form	HIV status	Diagnostic method	Treatment	Reference
1	3	F	Surat Thani, Thailand	VL	Negative	IFA, PCR	Pentamidine	31
2	40	M	Nan, Thailand	VL	Negative	Microscopy, PCR	Amphotericin	32
3	66	M	Bangkok, Thailand	VL	Negative	DAT, microscopy, PCR	Amphotericin	36
4	55	M	Phang Nga, Thailand	VL	Negative	DAT, microscopy, PCR	Amphotericin	15
5	37	M	Chanthaburi, Thailand	VL	Positive	Microscopy, PCR	Amphotericin	18
6	46	M	Songkhla, Thailand	CL and VL	Positive	Microscopy, PCR, culture	Amphotericin, itraconazole	19
7	30	M	Trang, Thailand	DCL and VL	Positive	Microscopy, PCR	Amphotericin, itraconazole	19
8	32	F	Trang, Thailand	DCL and VL	Positive	Microscopy, PCR, culture	Amphotericin, itraconazole	14
9	3	F	Lopburi, Thailand	CL	Negative	Microscopy	Itraconazole	34
10	45	F	Chiang Rai, Thailand	CL, VL	Positive	PCR	No treatment	20
11	34	M	Yangon, Myanmar	CL	Positive	PCR	Amphotericin	20
12	22	F	Yangon, Myanmar	Asymptomatic	Negative	PCR	No treatment	20
13	60	M	Yangon, Myanmar	DCL	Negative	Microscopy, PCR	Amphotericin	23
14	5	F	Satun, Thailand	VL	Negative	DAT, microscopy, PCR	Amphotericin	22
15	52	M	Lamphun, Thailand	VL	Negative	Microscopy, PCR	Amphotericin	27
16	48	M	Chiang Mai, Thailand	DCL	Positive	Microscopy, PCR	Amphotericin, itraconazole	28
17	38	M	Lamphun, Thailand	DCL	Positive	Microscopy, PCR	Amphotericin, itraconazole	28
18	28	F	Songkhla, Thailand	Asymptomatic	Positive	PCR	No treatment	29

CL = cutaneous leishmaniasis; DAT = direct agglutination test; DCL = diffused cutaneous leishmaniasis; F = female; HIV = human immunodeficiency virus; IFA = indirect immunofluorescence assay; M = male; PCR = polymerase chain reaction; VL = visceral leishmaniasis.

bleeding gums and epistaxis. Gastrointestinal symptoms were also observed in some cases. Laboratory findings showed pancytopenia and hyperglobulinemia. Similar clinical pictures were also found in a person with VL caused by *L. siamensis*.

SANDFLY DISTRIBUTION AND POTENTIAL VECTOR

A few surveys of sandfly species were conducted in different parts of Thailand.[37–47] A recent review of sandfly distribution in Thailand indicated that at least 27 species of the four genera, *Sergentomyia*, *Phlebotomus*, *Idiophlebotomus*, and *Chinius* were identified.[42,43] Certain species such as *Sergentomyia gemmea* are the most predominant species in the north[33] as well as in the south.[24,42,44] The prevalence of *S. gemmea* was as high as 85–95% in the northern and southern areas. However, almost 50% of limestone cave-dwelling sandfly species in a hilly area in the north was *Nemopalpus vietnamensis*, of which *Sergentomyia* species was found less than 1%.[41]

The studies of potential vectors were conducted around the affected areas in southern provinces.[24,42] Using the PCR-ITS1 and PCR-*hsp*70, DNA of *Leishmania* was detected in *Sergentomyia* (*Neophlebotomus*) *gemmea* and *Sergentomyia* (*Parrotomyia*) *barraudi*. As shown in Table 2, analysis of nucleotide sequence compared with the reference GenBank database of ITS1 (JQ866907) and *hsp*-70 (JX852708) revealed that *S. gemmea* and *S. barraudi* could serve as potential vectors of *L. martiniquensis* in the south.[24,25] Studies of the natural sandfly vector are very important for control disease transmission; thus, large-scale surveys of sandfly species in affected areas are needed. Due to limited knowledge on vector competency of *Sergentomyia*, more studies are required on their host-preference behavior and blood meal feeding including their breeding habitat.

ANIMAL RESERVOIRS

Transmission of leishmaniasis in Thailand is most likely to be a zoonotic cycle, in which animal reservoirs play an important role for disease transmission in nature. Recently, DNA of *L. martiniquensis*, previously identified as *L. siamensis* lineage PG[25] was detected in the blood, liver, and spleen of black rats (*Rattus rattus*) captured around the patients' house, which could serve as a natural reservoir host. Moreover, evidence of zoonotic transmission has been demonstrated when CL caused by *L. martiniquensis* was reported in farm animals, that is, horses in Germany,[16] bovines in Switzerland,[17] and one horse in Florida,[21] of which sequence analysis of the ITS1 of the SSU-rRNA gene of *Leishmania* DNA was similar to the species of *Leishmania* reported in Thailand.[15] Thus, zoonotic transmission of leishmaniasis in Thailand could be similar to that of *L. infantum* in Europe and the Mediterranean Basin.

CONCLUSION

The recent reported leishmaniasis cases raise awareness among clinicians and public health personnel, as well as the need for public alertness concerning this emerging disease in Thailand. The current evidence has confirmed two species of causative agents, that is, *L. martiniquensis* and *L. siamensis*, which are closely related to *Leishmania enrietti* complex. *L. martiniquensis* infection in humans and animal populations has been reported from different geographical areas, that is, the Caribbean Island, Central Europe, Florida, Thailand, and Myanmar, whereas *L. siamensis* infection was only reported in one Thai patient with AIDS.

The information on biology and epidemiology regarding these two species is still lacking. True prevalence, incidence, and risk factors of the disease especially in a high-risk, HIV/AIDS population need to be identified. In addition, studies of natural vectors and reservoir hosts are very important to prevent and control disease transmission.

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