

Case Report: Simple Nodular Cutaneous Leishmaniasis Caused by Autochthonous *Leishmania (Mundinia) orientalis* in an 18-Month-Old Girl: The First Pediatric Case in Thailand and Literature Review

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Abstract. We report an autochthonous case of simple, localized cutaneous leishmaniasis in a healthy 18-month-old girl from southern Thailand. The patient presented with a solitary chronic cutaneous nodular lesion on her left cheek for approximately 1 year. Histopathological dissection of the cheek skin biopsy demonstrated remarkably nodular and interstitial infiltrates of lymphocytes and histiocytes full of intracellular oval-shaped amastigotes, consistent with cutaneous leishmaniasis. The *Leishmania* promastigotes were also cultured successfully from the lesion biopsy and were designated with the WHO code MHOM/TH/2021/CULE5. Using internal transcribed spacer 1-specific polymerase chain reaction, the parasite DNA was demonstrated in both saliva and lesion biopsy. Based on the BLASTn and phylogenetic analysis, the parasite was identified as *Leishmania orientalis*, clustered in the *Mundinia* subgenus. The patient responded well to a 6-week course of oral itraconazole, without recurrence. To our knowledge, this is the fourth case of autochthonous leishmaniasis resulting from *L. orientalis* and the youngest patient of leishmaniasis ever reported in Thailand. More importantly, we also demonstrate the clinical course of the lesion according to the timeline before and after treatment, which can help physicians better understand and provide an accurate diagnosis with appropriate treatment of this emerging parasitic disease.

INTRODUCTION

Human leishmaniasis is a neglected infectious disease that infects more than 1 million new cases worldwide annually.¹ Approximately 53 *Leishmania* species have been recorded, 20 of which are known to be pathogenic to humans.^{2,3} *Leishmania* parasites have been known to be transmitted principally by phlebotomine sand flies.⁴ The spectrum of disease can be categorized broadly into three main forms: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis, and visceral leishmaniasis (VL).⁵ In Thailand, leishmaniasis is considered an emerging infectious disease because autochthonous leishmaniasis cases have increased continuously. The majority of cases are reported in the northern and southern provinces of the country, most frequently diagnosed with *Leishmania martiniquensis* and *Leishmania orientalis*.^{5,6} These two emerging *Leishmania* species have been classified recently in the same subgenus *Mundinia* with *Leishmania* sp. *Ghana*, *Leishmania enriettii*, and *Leishmania macropodum*.^{7–11} We describe the clinical presentation of autochthonous, localized CL caused by recently emerging *L. orientalis* in the youngest pediatric patient ever recorded in Thailand and compile all preceding information on indigenous leishmaniasis resulting from *L. orientalis*.

CASE DESCRIPTION

An 18-month-old Thai girl presented with a chronic, painless cutaneous nodule on her left cheek for ~1 year. She was previously healthy with no known underlying disease. Initially,

her mother noticed an erythematous papule on her left cheek when she was 6 months old. The papule gradually enlarged and transformed into a nodule. There was no history of travel outside Thailand before the appearance of the lesion. The patient was born and had lived in Phipun District, Nakorn Si Thammarat, southern Thailand until the age of 6 months, and then moved to Phuket. She had been evaluated several times at local private clinics and hospitals in Phuket; however, only a topical steroid cream, topical calcineurin inhibitors, and topical antibiotics were prescribed with no clinically significant improvement. On September 30, 2021, she visited Vachira Phuket Hospital with a 4 × 6 mm cutaneous erythematous nodule on her left cheek (Figure 1A). On October 15, 2021, a skin biopsy was performed and showed a dense, diffuse lymphohistiocytic infiltrate with numerous, small yeast-like intracellular organisms predominantly in the superficial dermis, and mild acanthosis with reactive changes at the overlying epidermis. CL and deep fungal infection, including penicilliosis, and histoplasmosis, were suspected. She was then referred to King Chulalongkorn Memorial Hospital in Bangkok for further investigations. Upon arrival at King Chulalongkorn Memorial Hospital November 23, 2021, a physical examination revealed the well-defined, solitary erythematous violaceous nodule and central overlying yellowish crust with a size of 5 × 7 mm on her left cheek (Figure 1B and C). No skin lesions in other sites were observed. Other examinations were within normal limits, including no fever, no lymphadenopathy, and no hepatosplenomegaly. Complete blood counts showed a hemoglobin level of 12.8 g/dL, a white blood cell count of 9,550 cells/mm³ (neutrophils, 33%; lymphocytes, 61%; monocytes, 4%; and atypical lymphocytes, 2%), and a platelet count of 414,000/mm³. Her HIV serology was negative. On December 7, 2021, an excisional biopsy of the lesion was performed. The histopathological examination was repeated

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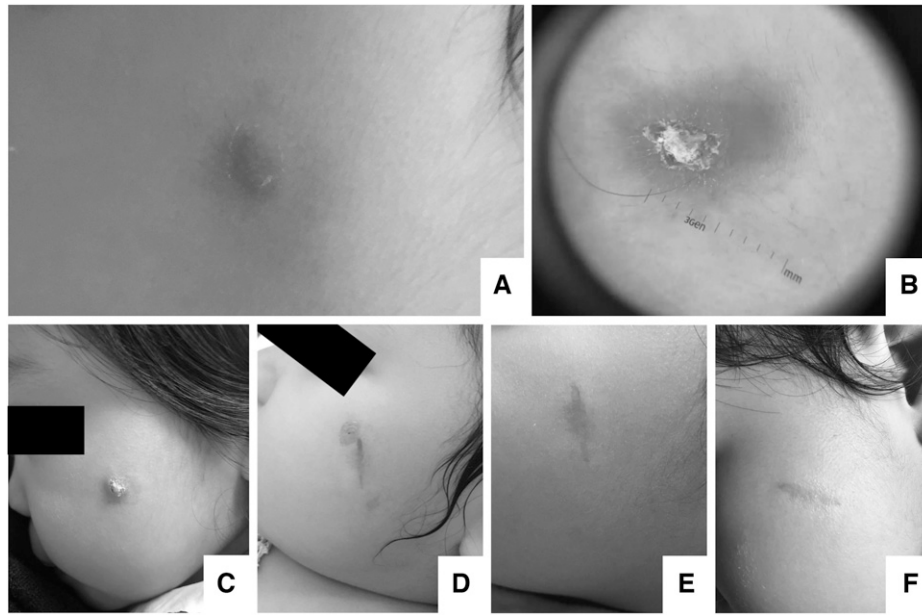


FIGURE 1. (A) A cutaneous erythematous nodule on the left cheek in October 2021. (B, C) When referred to King Chulalongkorn Memorial Hospital, a well-defined, cutaneous, nodular and centrally crusted lesion with an irregular border was observed. Skin appearance 20 days postexcision (D), 48 days postexcision and 20 days after starting itraconazole (E), and 2 months postexcision and after completing a 6-week course of oral itraconazole (F). This figure appears in color at www.ajtmh.org.

(Figure 2) and showed the same findings as described previously by Vachira Phuket Hospital. In addition, the periodic acid–Schiff-, Gomori methenamine silver-, acid-fast bacilli-, and modified acid-fast bacilli-stained sections were negative for organisms. Cultures for *Mycobacterium* and fungus were also negative. Furthermore, the whole blood, saliva, and skin biopsy specimens were investigated for molecular diagnosis and *Leishmania* cultivation. Genomic DNA was extracted from the lesion biopsy, saliva, and whole blood specimens. Polymerase chain reaction (PCR) was conducted using specific primers (LeF: 5'-TCCGCCCGAAAGTTCACCGATA-3' and LeR: 5'-CCAAGTCATCCATCGCGACACG-3') with thermocycler condition reported previously¹² to amplify a product of

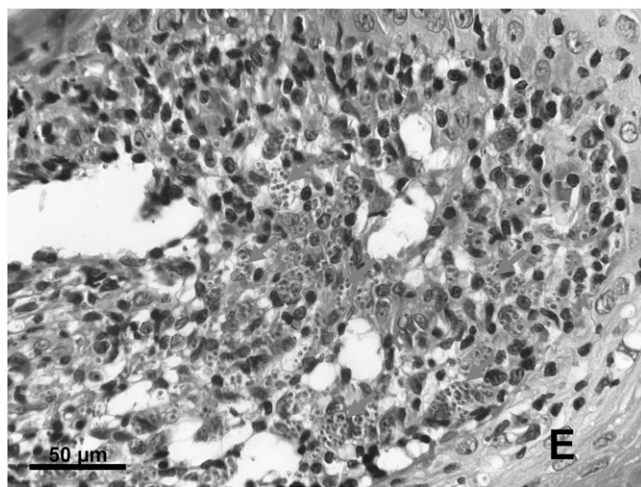


FIGURE 2. Hematoxylin and eosin-stained section of the skin biopsy showing numerous intracellular amastigotes of *Leishmania* parasites in tissue macrophages (arrows), surrounded by several inflammatory cells in the upper dermis. E = epidermis. This figure appears in color at www.ajtmh.org.

371 bp spanning the internal transcribed spacer 1 (*ITS1*) with 18S and 5.8S ribosomal DNA flanking regions. Amplification was successful in biopsy and saliva specimens, but negative for whole blood (Figure 3). Direct sequencing and the BLASTn alignment were done, revealing that the obtained sequence was 100% identical to *L. orientalis* MHOM/TH/2010/PCM2 Trang (accession no. JX195640). The *ITS1* sequence from this case was deposited in GenBank with accession no. ON303842. *Leishmania orientalis* promastigotes were cultured successfully from the lesion biopsy, exhibiting stage morphological variations (Figure 4). The culture isolate was assigned the WHO code MHOM/TH/2021/CULE5. The *ITS1* phylogeny was also studied (Figure 5). Last, a definite diagnosis of localized CL resulting from *L. orientalis* was established. The patient was then treated with oral itraconazole 10 mg/kg/d for 6 weeks, with clinical improvement (Figure 1D–F). As of 4 months of follow-up, no relapse has been observed.

DISCUSSION

Leishmania parasites are neglected pathogens that cause leishmaniasis in humans and animals worldwide. Since 1996, autochthonous leishmaniasis has been reported with increasing frequency. So far, more than 20 cases have been recorded, predominantly in the northern and southern provinces of Thailand. The two most prevalent *Leishmania* species in these indigenous cases were *L. martiniquensis*, and *L. orientalis*, respectively. Only one VL case resulting from *L. infantum* was reported in an HIV-seronegative male patient in 2008.¹³

We diagnosed an autochthonous case of localized CL in an 18-month-old Thai girl using *Leishmania*-specific PCR and in vitro parasite cultivation. Our study revealed that the multicopy *ITS1* amplification and DNA sequencing analysis can greatly facilitate the definitive diagnosis of leishmaniasis.

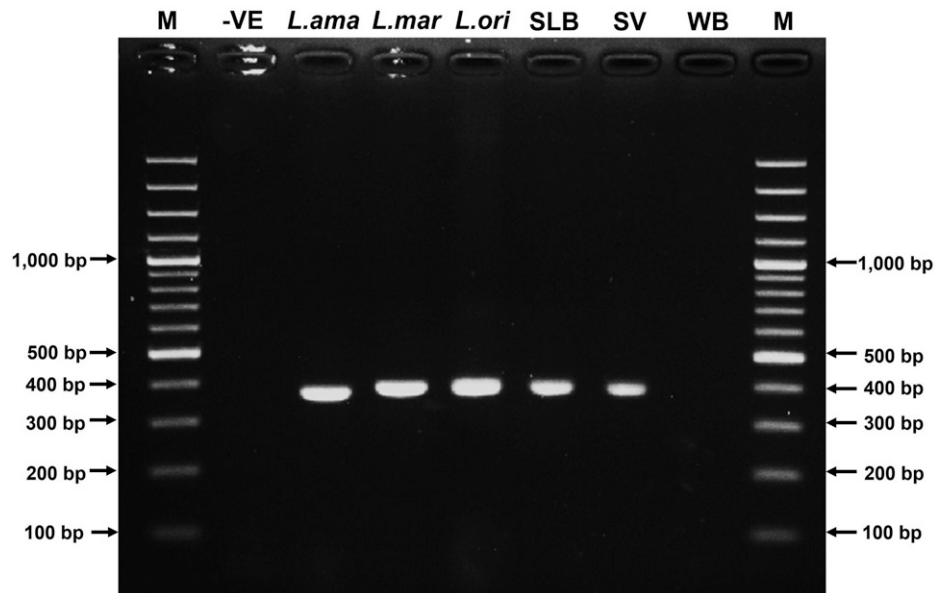


FIGURE 3. Internal transcribed spacer 1 polymerase chain reaction products. Amplicon sizes are 362 to 379 bp. Lanes 1 through 9 are, from left to right, 100-bp ladder marker (M), negative control (-VE), *Leishmania amazonensis* MHOM/BR/1997/M2269 (*L.ama*, 362 bp), *Leishmania martiniquensis* MHOM/TH/2012/CULE1 (*L.mar*, 379 bp), *Leishmania orientalis* MHOM/TH/2014/LSCM4 (*L.ori*, 369 bp); skin lesion biopsy (SLB, 371 bp), saliva (SV, 371 bp), whole blood (WB, no band), and 100-bp ladder marker (M).

Based on the BLASTn and phylogenetic inference, the causative parasite was identified as *L. orientalis*. Interestingly, the histopathological examination in this case apparently exhibited a chronic, intense inflammatory infiltrate of lymphocytes and macrophages full of amastigote forms. Similar

histopathological findings have also been reported in CL caused by other members of the *Mundinia* subgenus, including *L. martiniquensis*, and *L. enriettii*.^{9,14–18} Thus, it seems most likely that a remarkable feature of these *Mundinia* species is their ability to cause heavily parasitized macrophages,

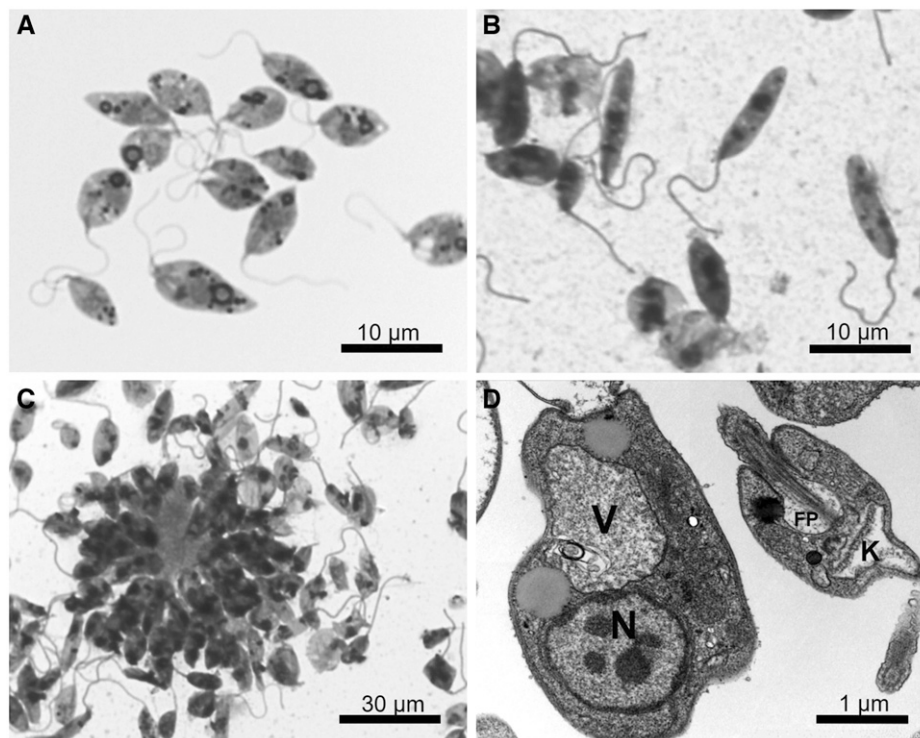


FIGURE 4. Giemsa-stained *Leishmania orientalis* MHOM/TH/2021/CULE5 promastigotes from a culture of the patient's biopsy tissue. (A) Procyclic form. (B) Leptomonad and nectomonad forms. (C) Rosette form. (D) Transmission electron micrograph showing the ultrastructure of the promastigotes. FP = flagellum within the flagellar pocket; K = kinetoplast; N = nucleus; V = large vacuole. This figure appears in color at www.ajtmh.org.

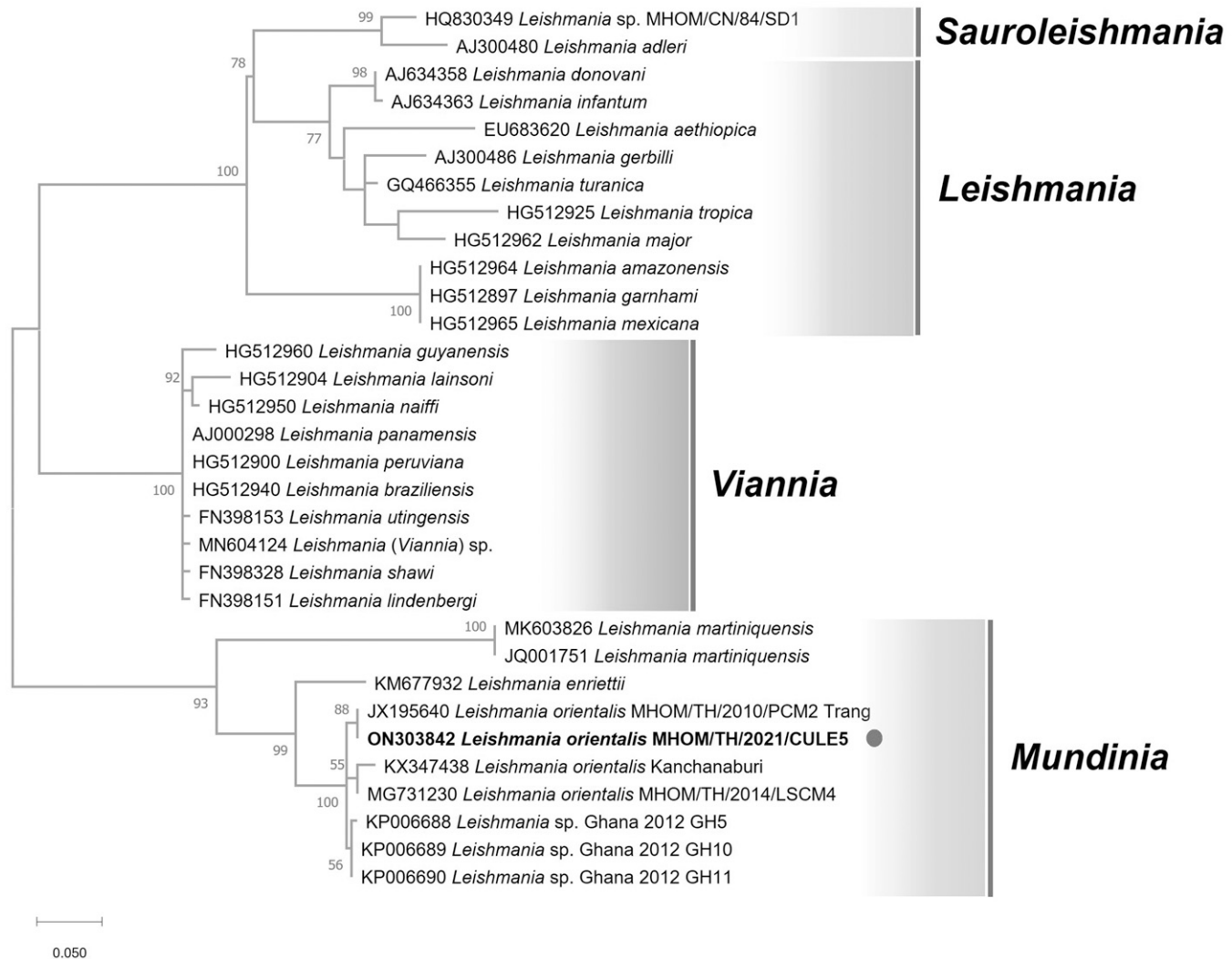


FIGURE 5. Molecular identification of the *Leishmania* clinical isolate as a member of the subgenus *Mundinia*. Maximum likelihood phylogeny of internal transcribed spacer 1 sequences from 32 *Leishmania* spp. isolates from four subgenera. Bootstrap branch support values greater than 50% are shown. The solid red circle indicates our patient. This figure appears in color at www.ajtmh.org.

compared with *Viannia* and *Leishmania* subgenera.^{9,14–19} This patient was then considered the fourth leishmaniasis case caused by autochthonous *L. orientalis*, and the leishmaniasis patient with the youngest age reported thus far in Thailand.

In addition to the biopsy, we demonstrated, for the first time, the molecular detection of *L. orientalis* DNA using a saliva sample. As published previously,²⁰ saliva has been reported as a promising clinical specimen for subclinical *L. martiniquensis* detection before the onset of symptoms. In addition, saliva can be used as an alternative source of parasite DNA for diagnosing molecularly malaria caused by *Plasmodium falciparum* and *Plasmodium vivax*.^{21,22} Therefore, saliva-based detection can be recommended as a rapid and noninvasive diagnostic test for disease surveillance and monitoring of emerging leishmaniasis in Thailand.

As reported previously^{6,23,24} in 2010, 2017, and 2018, the clinical presentations of leishmaniasis caused by *L. orientalis* could range from localized cutaneous to disseminated cutaneous, to visceral forms (Table 1). For the first two cases reported in 2010 and 2017, the parasites were formerly

identified as *Leishmania siamensis* and designated as PCM2 Trang, and an anonymous isolate from Kanchanaburi, respectively. Although *L. siamensis* has been mentioned in several previous publications, this name was considered an invalid name because of the lack of formal parasite characterization.^{6,8} In 2018, *Leishmania* sp. strain LSCM4, isolated from a patient in Nan Province in 2014, was shown to be very similar genetically to the other two previous cases (PCM2 Trang and the anonymous isolate from Kanchanaburi), and thus was formally described with the taxonomic characterization as *Leishmania* (*Mundinia*) *orientalis*.⁶ Although its nomenclature refers to its eastern origin, *L. orientalis* has been reported exclusively in Thailand to date. Based on the *ITS1* phylogeny, all four isolates of *L. orientalis*, including that in our patient, were identified as members of the same *Mundinia* subgenus as the other four *Leishmania* species (Figure 5).

Interestingly, all four *L. orientalis* isolates were also clustered in the phylogenetic tree closely with *Leishmania* sp. Ghana, which is responsible for localized CL in Ghana, West Africa. The existence of two closely related allopatric *Leishmania* species in different geographic localities strongly supports the

TABLE 1
Clinical information on autochthonous leishmaniasis cases resulting from *Leishmania orientalis* in Thailand from 2010 until 2021

Patient no.	Year	Locality	Gender	Age, years	AIDS, CD4 count	Species	Manifestations	Treatment	Relapse	Reference
1	2010	Trang	Female	32	Yes, CD4 = 107 cells/mm ³	<i>L. orientalis</i> MHOM/TH/2010/PCM2 Trang*	DCL (face, trunk, and legs), VL (severe anemia and generalized hepatomegaly)	There was no treatment because the patient died after 2 weeks from a seizure before amphotericin B administration	N/A	Bualert et al. ²³
2	2014	Nan	Female	57	No	<i>L. orientalis</i> MHOM/TH/2014/LSCM4	CL (left cheek and left mouth angle)	Oral amphotericin B (1 mg/kg/d for 1 day) and fluconazole (200 mg/kg/d for 45 days)	No	Jariyapan et al. ⁶
3	2015	Kanchanaburi	Female	42	Yes, CD4 = 89 cells/mm ³	<i>L. orientalis</i> anonymous isolate* (genetically identical to PCM2 Trang)	DCL (face, trunk, and both legs)	First intravenous amphotericin B (0.6 mg/kg/d for 4 weeks) and then additional intravenous amphotericin B (total dose, 1,260 mg), followed by itraconazole 400 mg/d until improved	No	Suprisunjai et al. ²⁴
4	2021	Nakhon Si Thammarat	Female	1½	No	<i>L. orientalis</i> MHOM/TH/2021/CULE5	CL (left cheek)	Excisional biopsy and oral itraconazole (10 mg/kg/d for 6 weeks)	No	Our report

CL = cutaneous leishmaniasis; DCL = disseminated cutaneous leishmaniasis; N/A = not available; VL = visceral leishmaniasis.

* Species names identified previously as *Leishmania siamensis* and named retrospectively *L. orientalis*, with formal characterization in 2018.

hypothesis of supercontinent origins for the genus *Leishmania* before the breakup of Gondwana ~200 million years ago.^{25,26} Therefore, phylogenomic analysis of the African and Asian *Mundinia* isolates will shed light on this speculation. In addition, most Ghanaian CL cases had a single lesion, and more than half the lesions were in the head region, similar to localized cutaneous presentation resulting from *L. orientalis* in our patient, in which the single lesion was located on the facial area. It has been noted⁸ that the head is typically an exposed area, especially at nighttime, and is more prone to a vector's bite than other parts of the body.

From the clinical history known, our patient most likely acquired the infection before leaving Nakhon Si Thammarat, which is supported by the identical homology between the obtained *ITS1* sequence with that of the PCM2 Trang isolate retrieved previously from the neighboring Trang Province. However, such two genetically similar isolates expressed different disease severity in patients with different immune statuses (Table 1). In addition, it is likely to have a greater degree of virulence in immunocompromised patients, with more severe manifestations, such as disseminated CL and VL. In contrast, mild CL was diagnosed in the non-HIV patients, with only a simple nodular lesion, as in our patient. Of note, CL should be added to the list of differential diagnoses for a chronic nodular lesion, especially in the leishmaniasis foci of the country.

The increasing incidence of autochthonous leishmaniasis cases in Thailand emphasizes the importance of identifying natural vectors and animal reservoirs of the parasites, which remain unclear. The DNA of *L. orientalis* (previously *L. siamensis*) was detected by heat shock protein 70-specific PCR in one female *Sergentomyia iengari* sandfly collected around a VL patient's house in Trang Province.²⁷ In addition, the molecular detection of *L. orientalis* has been recently reported in *Sergentomyia gemmea*, collected from Chiang Rai Province, northern Thailand.²⁸ However, a recent entomological investigation and phylogenetic analysis of the mitochondrial cytochrome b gene revealed that the morphological identification of *Sergentomyia* species is complicated, suggesting systematic revision for these potential vectors.²⁹ More surprisingly, it was shown that *L. orientalis* promastigotes can develop in laboratory-reared biting midges (*Culicoides sonorensis*) and that *C. sonorensis* can transmit *L. martiniquensis*, *L. orientalis*, and *Leishmania* sp. *Ghana* to BALB/c mice in the laboratory.^{30,31} These findings implicate biting midges of the genus *Culicoides* as putative vectors in the transmission of human pathogenic *Leishmania* (*Mundinia*) species. Recently, *L. martiniquensis* DNA detection in biting midges *Culicoides mahasarakhamense*, collected from an affected area in Lamphun Province, northern Thailand, has been reported.³² Therefore, the species diversity of such hematophagous dipteran insects and the evidence of vector incrimination in different regions of Thailand, especially in the northern and southern provinces, need to be explored further. Essentially, the clinical isolates of autochthonous *L. orientalis* parasites need to be investigated to provide more biologic information about their differential virulence and to help us understand these emerging parasites more thoroughly.

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