

Clinical report

Cutaneous myiasis caused by *Dermatobia hominis* in Thai travelers: first report in Thailand

Apichart Thanapatcharoen^a, Kanok Preativatanyou^b, Atchara Phumee^c, Kanyarat Kraivichain^b, Prasert Sitthicharoenchai^b, Henry Wilde^d, Padet Siriyasatien^b

^aSamitivej Sukhumvit Hospital, Bangkok 10110, ^bDepartment of Parasitology, ^cMedical Science Program, ^dDivision of Infectious Diseases, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand

Background: Myiasis is the infestation with fly larvae in live vertebrate hosts. The disease has not been reported in Thailand.

Method and Results: We report the first case series of cutaneous myiasis caused by *Dermatobia hominis* in two Thai travelers who visited Brazil. Two of five travelers were infested with *D. hominis* larvae. Both presented with furuncular lesions. Surgical excision was performed for both patients and the larvae were removed. They were identified as second stage of *D. hominis*. Sequence data of both mitochondrial and nuclear genes of the larva were similar to previous reports from Brazil.

Conclusion: With increasing travel into endemic countries of *D. hominis*, physician should be aware of this parasitic infestation.

Keywords: *Dermatobia hominis*, imported case series, Myiasis, Thailand

Myiasis is the infestation with fly larvae in live vertebrate hosts. *Dermatobia hominis* (human botfly) is an obligate parasite that infests man and animals including live stock and other domestic animals [1]. Additionally, it is the most common cause of cutaneous myiasis in man [2]. *D. hominis* occurs in Central and South American countries. Adult *D. hominis* has a vestigial mouth, it cannot feed and can survive for only a few days. During this short period of life, adult females deposit packets of eggs on other arthropods (called phoretic hosts) which include haematophagous arthropods such as day-flying mosquitoes (*Psorophora*), flies (*Stomoxys*), ticks (*Amblyomma*) or event on non-blood feeding flies (*Sarcophaga* and *Musca*) [3]. Eggs are activated to hatch by the body heat of the vertebrate hosts when the phoretic host feeds or lands on a vertebrate animal [4]. The larvae then invade the vertebrate host either through a hair follicle, bite or by directly burrowing into the host's skin within five to ten minutes [3, 5]. After 6 to 12 weeks of full larval development, the mature larva emerges from the host's skin and drops

to the ground for pupation. It finally develops into an adult fly and the cycle can then repeat [6]. This type of insect infestation can be found in Central and South American countries, e.g. Brazil. However, there is yet no previous report from Thai travelers who visited and returned infested. Herein, we report the first case series of imported cutaneous myiasis in Thailand from two travelers returning from Brazil. This study also demonstrates the use of internal transcribed spacer 2 (ITS2) and mitochondrial cytochrome oxidase (COI-COII) gene sequences to provide DNA-based identification and confirm whether they were indeed infested in Brazil.

Case report

A 33 (case 1) and a 43 (case 2) year old men traveled to Brazil together. They noticed small papules on their left arm two days before they left Brazil to return to Thailand. One week after arrival, they observed that the lesions became enlarged and inflamed. After taking an oral antibiotic for a week, they found that the lesions did not improve and there was serosanguineous exudate excreting from a central pore of the lesion. They presented to a private hospital. The initial diagnosis was foreign body abscess.

Correspondence to: Padet Siriyasatien, Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. E-mail: Padet.S@chula.ac.th

Case 1

The furuncular lesion was located at the ventral side of the left forearm with a central pore. The size of the lesion was approximately 2×1.5 cm. Surgical excision was performed under local anesthesia (**Figure 1A**). The bottle-neck shaped larva, approximately 7×5 mm, was recovered. The posterior end of the larva was cut during the operation. Strong spines around the body of the larva could be observed macroscopically. The larva was identified as a second larval stage of *D. hominis* (**Figure 1B**). Molecular identification by sequence comparison is described below.

Case 2

The furuncular lesion was on the dorsal side of the left arm just above the elbow and approximately 1.5×1.5 cm in size (**Figure 2A**). Surgical excision was performed. A botfly larva was observed. The larva and soft tissue were removed (**Figure 2B**). The live larva was of bottle-neck shape, approximately 10×5 mm in size. The larva presented with many rows of backward projecting spines around the body (**Figure 3**). A prominent pair of cephalic hooks (**Figure 4A**) and posterior spiracles were seen (**Figure 4B**).

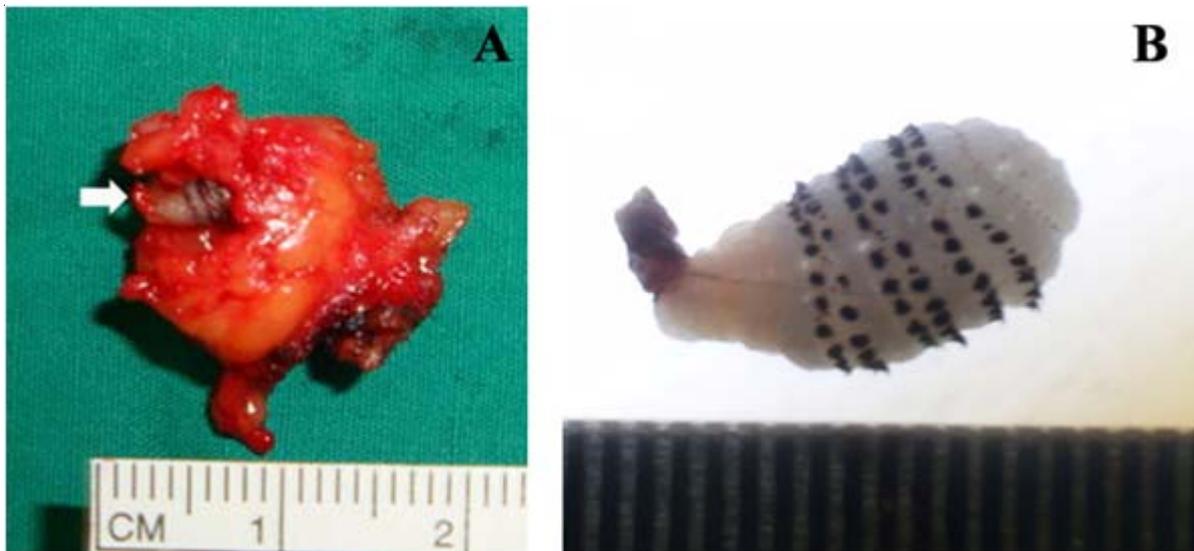


Figure 1. **A:** Surgical specimen from first case, a second stage larva of *D. hominis* (arrow). **B:** The larva of *D. hominis* from the first case. Tail of the larva was cut during the operation.

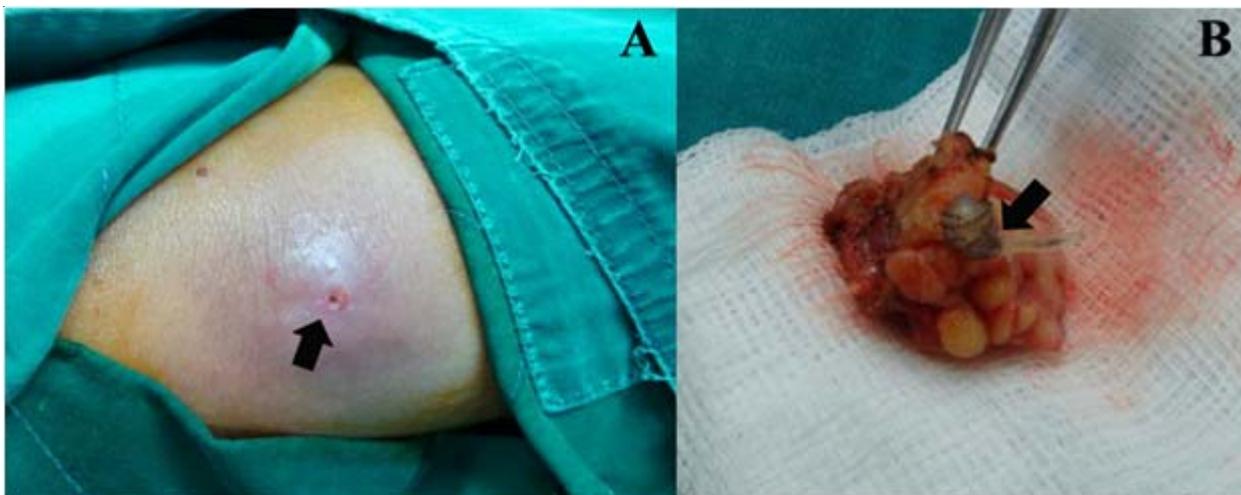


Figure 2. Furuncular lesion at ventral side of left forearm (**A**) with a central pore where serosanguineous exudates was drained (arrow). Surgical removal from second case (**B**), a live second stage larva of *D. hominis* (arrow).



Figure 3. Alive second larva of *D. hominis* from second case.

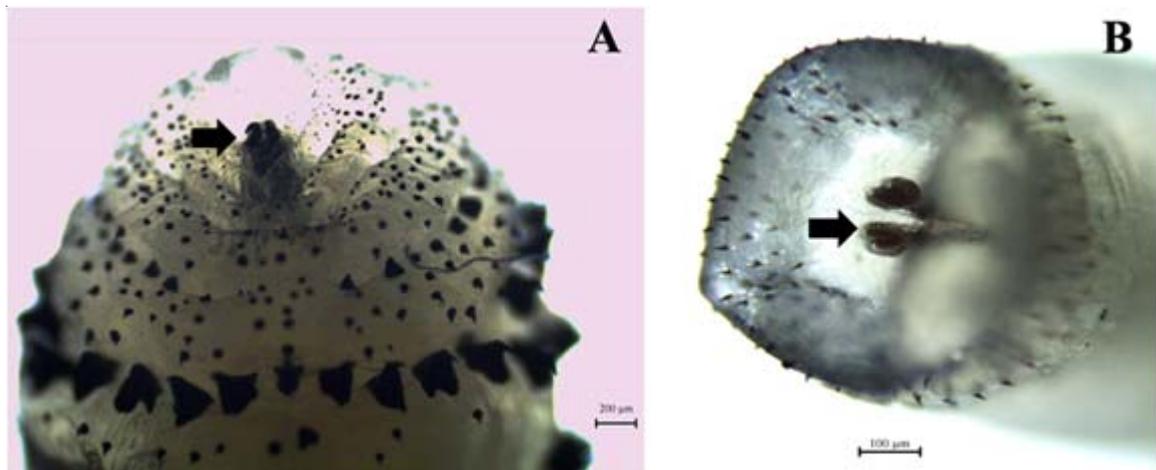


Figure 4. Anterior part of *D. hominis* larva (A), a pair of cephalic hooks (Arrow) and rows of backward projecting spines around the body. Posterior end of *D. hominis* larva (B), a pair of posterior spiracles (arrow).

Molecular Identification Methods

DNA extraction

Genomic DNA was extracted from the whole larva of the first case using QIAamp DNA mini kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's instructions. The eluted DNA was verified by spectrophotometer and diluted with 10 mM Tris-EDTA buffer, pH 8.0 to a concentration of 50 ng/µl and then stored at 4°C until the test.

PCR amplification and DNA sequencing

To retrieve sequence data of ITS2, two oligo sequences [7] as follows: 5'TGCTTGGACTACA TATGGTTGA3' and 5'GTAGTCCCATATGAGTTG

AGGTT3' were synthesized for forward and reverse primers, respectively. For COI-COII, the forward primer 5'CAGCTACTTTATGAGCTTTAGG-3' and reverse primer 5'GAGACCATTACTTGCTTTCAG TCATCT-3' were designed from previous studies [8, 9]. The PCR reactions were carried out in a GeneAmp PCR System 2400 thermal cycler (Applied Biosystem, Foster city, CA, USA) using the condition as follows: the initial denaturation (94°C for three minutes); followed by 30 cycles of 94°C for 45 seconds, 60°C (ITS2), or 56°C (COI-COII) for 45 seconds and 72°C for 1.0 minutes, and the final extension at 72°C for 10 minutes. The PCR amplification reaction was set up in a final volume of

25 μ l, containing 150 ng of extracted DNA, 0.4 mM of each primer, 2.5 mM of $MgCl_2$, 200 mM of dNTPs and 1 unit of *Taq* DNA polymerase (Fermentas, USA). The PCR products were detected in 1% agarose gel (**Figure 5**) and then purified by using a Perfectprep \square Gel Cleanup kit (Eppendorf, Germany) following the manufacturer's instructions. Direct DNA sequencing was performed with the primer pairs described previously (1st BASE Laboratories, Malaysia).

Sequence analysis

Nucleotide sequences were prepared using Chromas Lite version 2.01 (<http://www.technelysium.com.au>) and aligned for maximum score comparison by the BLAST programme in NCBI website (<http://www.ncbi.nlm.gov/BLAST>). Two identified sequences with related data from this study were submitted and assigned for accession numbers as HQ215834 for ITS2 and HQ334260 for COI-COII in the GenBank database. Sequences were also aligned and compared with sequences data obtained from GenBank and sequence identity was calculated by using the Clustal W algorithm implemented in the BioEdit Sequence Alignment Editor v. 6.0.7 (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) (**Figures 6 and 7**).

Result and discussion

Although myiasis caused by *D. hominis* has been reported in many parts of the world, including as imported cases in the United States [1, 5, 10], United Kingdom [11], the Netherlands [2], Austria [12], Italy [13], and Japan [14], it was never reported from South East Asian countries. Here we describe two cases of Thai travelers infested by *D. hominis*. Both patients developed typical signs of furuncular myiasis on their extremities. Although the larvae obtained by surgical removal were identified by morphological appearance, molecular study provided more information of the source of infestation by comparison with sequence data in GenBank. The primer sets used in this study have been successfully used for identification of other fly species of the Order Diptera (suborder Brachycera) [7, 8, 9, 15]. This report demonstrated the use of these primer sets to amplify the ITS2 and COI-II genes of *D. hominis*, and obtained expected PCR products of approximately 645 bp and 1300 bp for the ITS2 and COI-II genes respectively (**Figure 5**). Sequence analysis of the ITS2 and COI-II genes by the BLAST programme compared with the GenBank database, found that the percentage of similarity was equal to 99% with expected value = 0.0 for both ITS2 and COI-COII sequences. Thus, it could be concluded that the species of this larva was

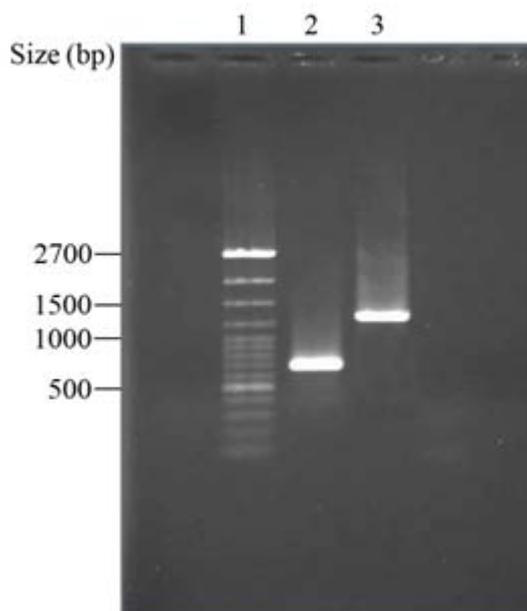


Figure 5. 1% agarose gel electrophoresis demonstrates the PCR products of amplified ITS2 and COI-II genes of *D. hominis*. Lane 1; 100bp marker (Fermentas, USA), lane 2, and lane 3; PCR products of amplified ITS2 and COI-II gene regions respectively.

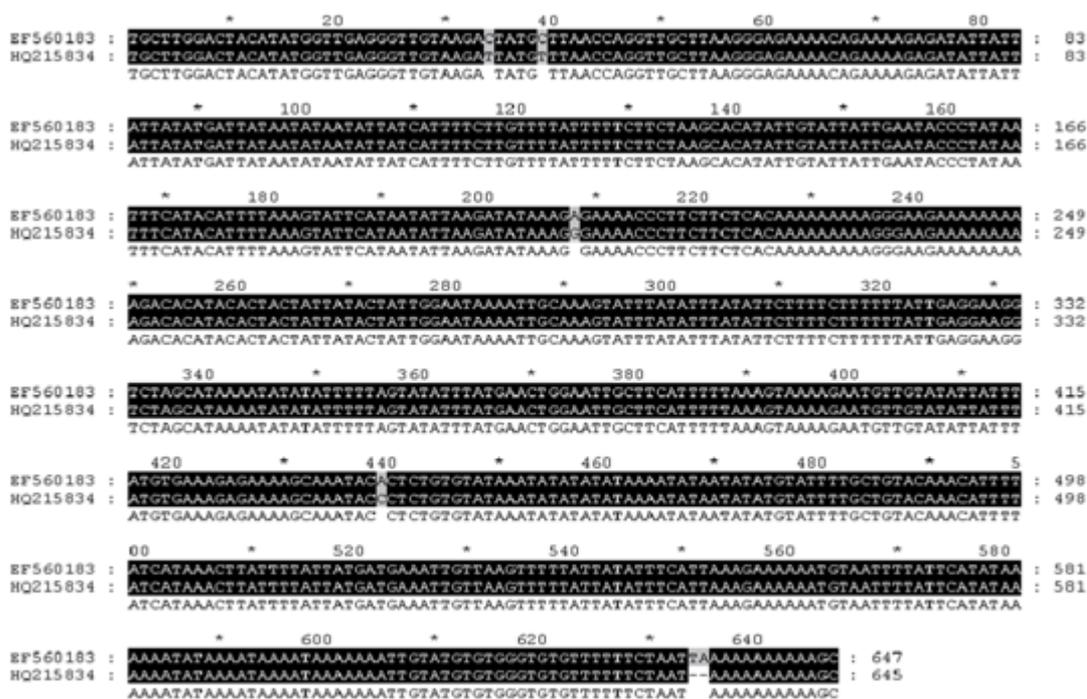


Figure 6. Nucleotide sequence alignment of *D. hominis* ITS2 gene from GenBank (EF560183) and *D. hominis* ITS2 gene from the patient (HQ215834).

D. hominis. This confirmed that this infection occurred in Brazil since the sequences are matched with GenBank sequence studied in Brazil (EF560183 for ITS2 and AY463155 for COI-COII) and confirm to the travel history to Brazil. Therefore, our data are the first report for Thai travelers infected with botfly myiasis and successfully show the application of molecular markers.

Myiasis is an infestation by fly larva in living mammals. Several fly species can cause furuncular myiasis in humans including *D. hominis* (human botfly) in Central and South American countries, *Cordylobia anthropophaga* (African tumbu fly) in Africa and *Hypoderma* spp. in North America, Europe, Africa and Asia [16]. *D. hominis* is an obligate parasite, all larval stages need to develop on a mammalian host, including humans. *Dermatobia*, *Hypoderma* and *Cordylobia* infection. They can cause skin lesions which may lead to misdiagnosis if this condition is not known to the local physician. Some studies stated that patients were usually given antibiotics or/and topical steroid cream without improvement [11, 14, 17]. The hallmark of this infection is a central pore and moving nodule. Apart from myiasis caused by *D. hominis* in the Americas,

another common cause of furuncular myiasis is the tumbu fly (*C. anthropophaga*) which predominates in continental Africa south of the Sahara. It causes boil-like (furuncular) lesions, similar to myiasis caused by *D. hominis* [3]. The life cycle of the tumbu fly has been documented by Blacklock and Thompson [18]. This parasite has a different life cycle to *D. hominis*. The female deposits batches of 200-300 eggs on dry, shaded ground often laid them on laundry being hung out to dry. Larvae hatch one to three days after been deposited, and survive for 9 to 15 days without food. The larvae then wait for a mammal, often man, to use the clothing and invade skin. One of the authors, while working in West Africa, encountered one expatriate with almost a dozen mature larvae in his groin region, underneath his underwear. His native servant instructed him to cover the “blow hole” in the center of the lesions with Vaseline. Within hours, the larvae had partly come out of the opening and could be easily extracted. The lesions then healed without complications within one week. No surgery or further treatment was necessary. It is therefore prudent not to hang laundry to dry outside in Africa. If this cannot be avoided, it must be aggressively ironed with a very hot iron. Many tourists from Asia are now known to

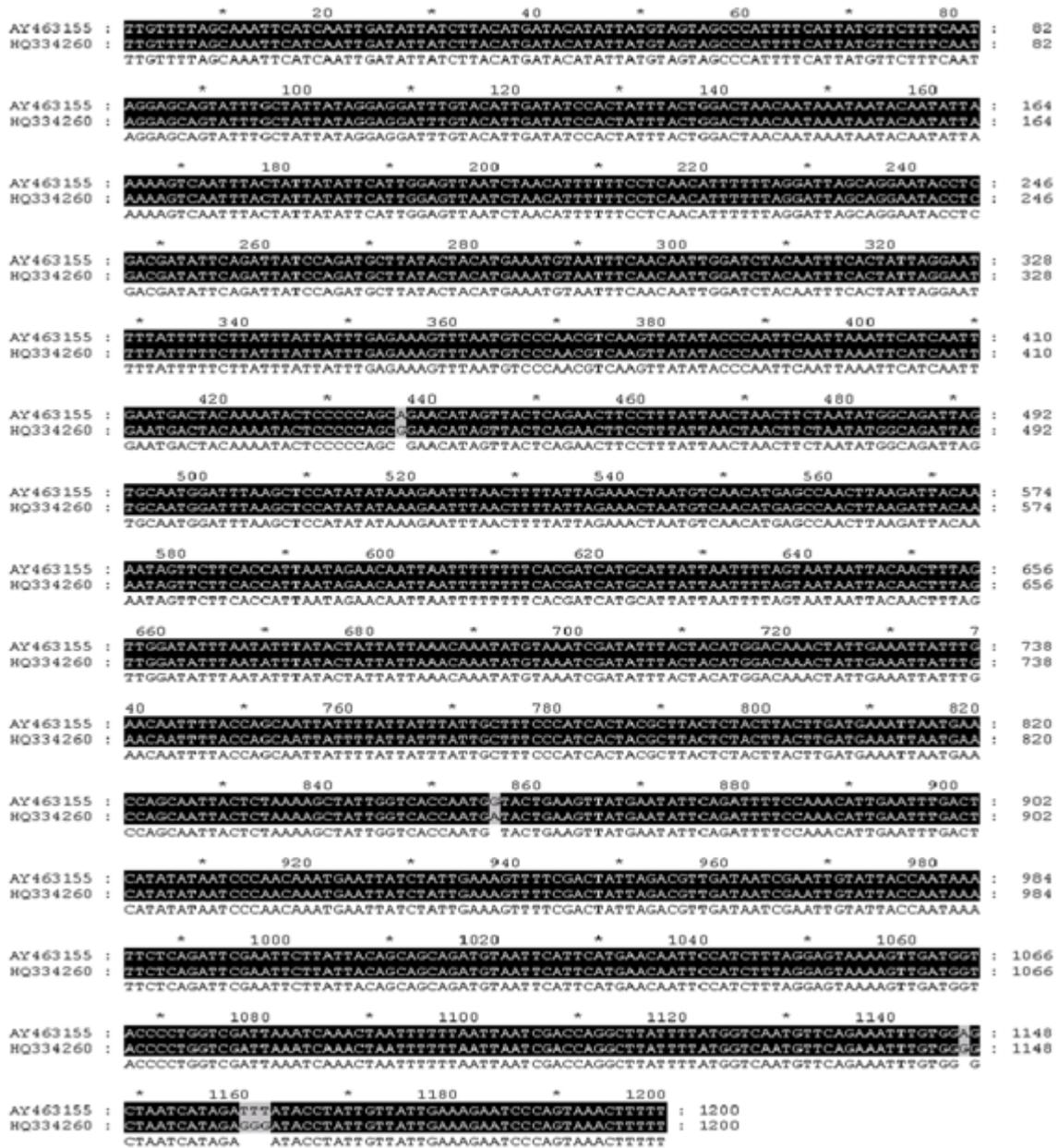


Figure 7. Nucleotide sequence alignment of *D. hominis* COI-COII gene from GenBank (AY463155) and *D. hominis* COI-COII gene from the patient (HQ334260).

travel to East and West Africa where tumbu fly myiasis is common. Although both bot fly and tumbu fly infestations show explicit furuncular lesions, differentiation between these two species is showed in Table 1.

Treatment of myiasis caused by *D. hominis* and *C. anthropophagi* is to remove the larvae from skin either by suffocating them using Vaseline and by squeezing the larvae out of the skin or by surgery.

Prophylaxis for travelers who are going into endemic areas is to avoid insect bites by using repellants in case of bot fly, and avoiding hanging laundry to dry outside in case of tumbu fly. Obtaining a travel history can help to establish a provisional diagnosis and may avoid surgery. Thai travelers to risk zones of infestation should take precautions against insect exposure and avoid exposing washed closing and themselves to flies.

Table 1. Comparison between *D. hominis* (bot fly) and *Cordylobia anthropophaga* (tumbu fly).

Fly Species	Endemic area	Number of lesion/case	Mode of transmission	References
<i>D. hominis</i> (bot fly)	Central and South Americas	One to few furuncular lesions	Indirect (Phoretic hosts)	[12, 19, 20, 21]
<i>Cordylobia anthropophaga</i> (tumbu fly)	Continental Africa south of the Sahara	Multiple furuncular lesions	Indirect (Orifice of burrows, clothing)	[12, 22, 23, 24]

Acknowledgements

This work was supported by Ratchadapisek Sompotch Fund, Faculty of Medicine, Chulalongkorn University and Ratchadapisek Somphot Foundation, Chulalongkorn University and the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission (HR1160A) and The Thailand Research Fund to W. Choochote. The authors have no conflict of interest to report.

References

- Cottom JM, Hyer CF, Lee TH. *Dermatobia hominis* (botfly) infestation of the lower extremity: a case report. *J Foot Ankle Surg.* 2008; 47:51-5.
- Missotten GS, Kalpoe JS, Bollemeijer JG, Schalijs-Delfos NE. Myiasis of the upper eyelid. *J AAPOS.* 2008; 12: 516-7.
- Hall MJR, Smith KJV. Diptera causing myiasis in man. In: Lane RP, Crosskey RW, editors. *Medical Insects and Arachnids*, Chapman&Hall, London, UK; 1993. p. 429-69.
- Gonçalves JM, Nascimento MF, Breyner NM, Fernandes VC, Gomes AM, Leite AC. Spleen cell proliferation during and after skin myiasis by human bot fly *Dermatobia hominis*. *Rev Inst Med Trop Sao Paulo.* 2009; 51:149-54.
- Bhandari R, Janos DP, Sinnis P. Furuncular myiasis caused by *Dermatobia hominis* in a returning traveler. *Am J Trop Med Hyg.* 2007; 76:598-9.
- Lane RP, Lowell CR, Griffiths WA, Sonnex TS. Human cutaneous myiasis: a review and report of three cases due to *Dermatobia hominis*. *Clin Exp Dermatol.* 1987; 12:40-5.
- Song ZK, Wang XZ, Liang GQ. Molecular evolution and phylogenetic utility of the internal transcribed spacer 2 (ITS2) in Calyptratae (Diptera: Brachycera). *J Mol Evol.* 2008; 67:448-64.
- Sperling FAH, Anderson GS, Hickey DA. A DNA-based approach to the identification of insect species used for postmortem interval estimation. *J Forensic Sci.* 1994;39: 418-27.
- Schroeder H, Klotzbach H, Elias S, Augustin C, Pueschel K. Use of PCR-RFLP for differentiation of calliphorid larvae (Diptera, Calliphoridae) on human corpses. *Forensic Sci. Int.* 2003; 132:76-81.
- Ofordeme KG, Papa L, Brennan DF. Botfly myiasis: a case report. *Can J Emerg Med.* 2007; 9:380-2.
- Messahel A, Sen P, Wilson A, Patel M. An Unusual case of myiasis. *J Infect Public Health.* 2010; 3:43-5.
- Maier H, Hognismann H. Furuncular myiasis caused by *Dermatobia hominis*, the human botfly. *J Am Acad Dermatol.* 2004; 50 (Suppl 2):S26-30.
- Calderaro A, Peruzzi S, Gorrini C, Piccolo G, Rossi S, Grignaffini E, Gatti S, Caleffi E, Dettori G, Chezzi C. Myiasis of the scalp due to *Dermatobia hominis* in a traveler returning from Brazil. *Diagn Microbiol Infect Dis.* 2008; 60:417-8.
- Nagamori K, Katayama T, Kumagai, A case of cutaneous myiasis due to *Dermatobia hominis* in Japan. *J Infect Chemother.* 2007; 13:255-7.
- Preativatanyou K, Sirisup N, Payungporn S, Poovorawan Y, Thavara U, Tawatsin A, Sungradit S, Siriyasatien P. Mitochondrial DNA-based identification of some forensically important blowflies in Thailand. *Forensic Sci Int.* 2010; 202:97-101.
- Logar J, Marinic-Fiser N. Cutaneous myiasis caused by *Hypoderma lineatum*. *Wien Klin Wochenschr.* 2008; 120:619-21.
- Passos MR, Ferreira DC, Arze WN, Silva JC, Passos FD, Curvelo JA. Penile myiasis as a differential diagnosis for genital ulcer: a case report. *Braz J Infect Dis.* 2008; 12:155-7.
- Blacklock B, Thompson MG. A study of the Tumbu fly *Cordylobia anthropophaga*, Grunberg in Sierra Leone. *Ann Trop Med Parasitol.* 1923; 17:443-502.
- Gordon PM, Hepburn NC, Williams AE, Bunney MH. Cutaneous myiasis due to *Dermatobia hominis*: a report of six cases. *Br J Dermatol.* 1995; 132:811-4.
- Guse ST, Tieszen ME. Cutaneous myiasis from *Dermatobia hominis*. *Wilderness Environ Med.* 1997; 8:156-60.

21. Vincent AL, Vicencio RP, Greene JN, Sandin RL, Sinnott JT. Botfly myiasis in a returning traveler. *Infect Med.* 2001; 18:163-6.
22. Baily GG, Moody AH. Cutaneous myiasis caused by larvae of *Cordylobia anthropophaga* acquired in Europe. *BMJ.* 1985; 290:1473-4.
23. Gunther S. Clinical and epidemiological aspects of the dermal tumbuflly myiasis in Equatorial-Africa. *Br J Dermatol.* 1971; 85:226-31.
24. Pampiglione S, Bettoli V, Cestari G, Staffa M. Furuncular myiasis due to *Cordylobia anthropophaga*, endemic in the same locality for over 130 years. *Ann Trop Med Parasitol.* 1993; 87:219-20.